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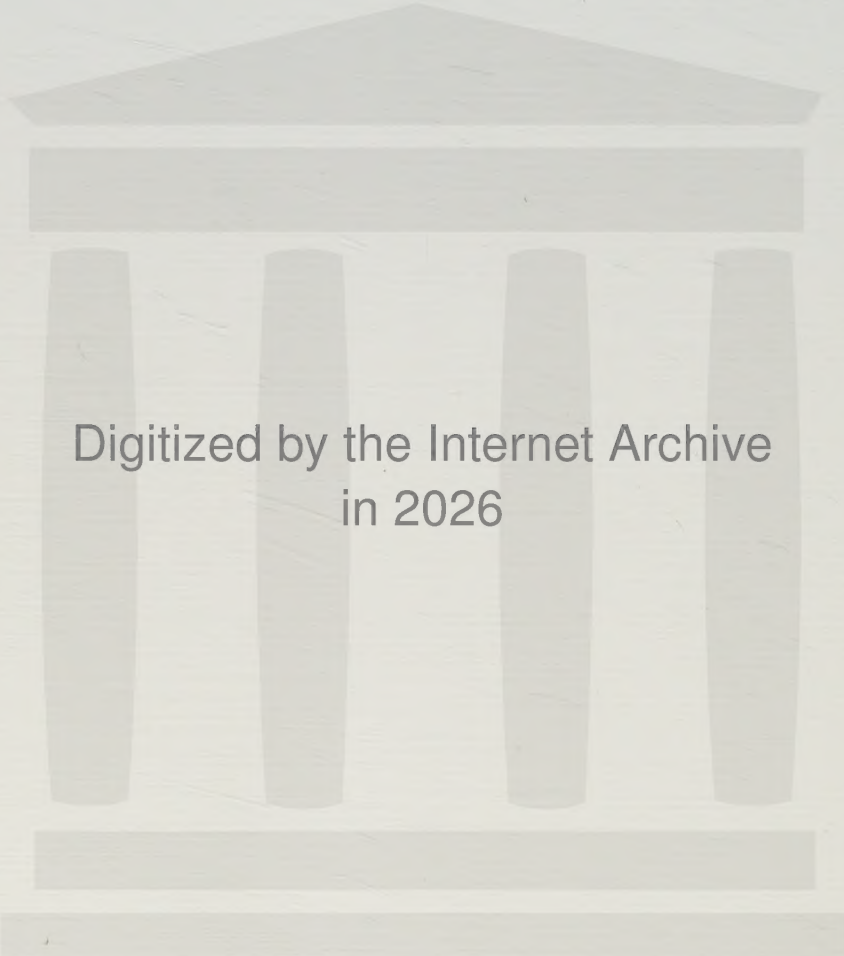
Supplementum 516

Beta₂-Adrenergic Control of
Transcapillary Fluid Absorption
and Plasma Volume in Hemorrhage

By

Jahn Hillman

Lund 1983



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From the Department of Physiology and Biophysics,
University of Lund, Sweden

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This thesis is based mainly on the following publications:

- I. LUNDVALL, J. & HILLMAN, J. 1978. Fluid transfer from skeletal muscle to blood during hemorrhage. Importance of beta adrenergic vascular mechanisms. Acta Physiol Scand 102:450-458.
- II. HILLMAN, J. 1981. Further studies on beta-adrenergic control of trans-capillary fluid absorption from skeletal muscle to blood during hemorrhage. Acta Physiol Scand 112:281-286.
- III. HILLMAN, J. & LUNDVALL, J. 1981. Hormonal and neurogenic adrenergic control of the fluid transfer from skeletal muscle to blood during hemorrhage. Acta Physiol Scand 112:271-280.
- IV. LUNDVALL, J., HILLMAN, J. & GUSTAFSSON, D. 1982. β -Adrenergic dilator effects in consecutive vascular sections of skeletal muscle. Am J Physiol 243 (Heart Circ Physiol 12):H819-H829.
- V. HILLMAN, J. & LUNDVALL, J. 1981. Classification of β -adrenoceptors in the microcirculation of skeletal muscle. Acta Physiol Scand 113:67-71.
- VI. HILLMAN, J., GUSTAFSSON, D. & LUNDVALL, J. 1982. β_2 -adrenergic control of plasma volume in hemorrhage. Acta Physiol Scand 116:175-180.

In the text, these papers are referred to by their Roman numerals.

INTRODUCTION

The stress imposed on the organism by hemorrhage is met by a number of rapid or more gradual compensatory defense mechanisms aimed at restoring cardiovascular homeostasis. The prompt reflex increase in sympathetic discharge and catecholamine release from the adrenals causes an almost instantaneous constriction of the capacitance vessels which tends to adapt the dimension of the circulatory system to the decreased blood volume. The concomitant reflex constriction of the resistance vessels and the cardiac excitation contribute importantly to recovery from the ensuing initial arterial hypotension. Such adjustments, however, do not guarantee a maintained steady state and the restoration of cardiovascular homeostasis is explicitly depending on the replacement of the shed blood volume. Otherwise the individual remains in a potential state of jeopardized tissue nutrition with secondary metabolic derangements and possible hypovolemic shock as a result. Various aspects of the latter topic have been extensively reviewed (e.g. Wiggers 1950, Lillehei et al. 1975, Messmer & Sunder-Plassman 1975, Schumer & Erve 1975, Zweifach & Fronek 1975, Haljamäe et al. 1979, Cowley & Trump 1982).

Recovery from hemorrhagic hypovolemia by physiological compensatory mechanisms requires both red cell and plasma volume restoration. Recruitment of red cells from the spleen is in some species an important means of causing a temporary increase in circulating cell volume, but the true restoration by increased erythropoiesis is a slow process. Early compensation for hypovolemia is therefore mainly depending on expansion of the plasma volume.

Previous investigations have revealed different mechanisms for such volume compensation, as reviewed, for instance, by Öberg 1964, Chien 1967, Folkow & Neil 1971, Fitzsimons 1972, Kovách 1973, Gauer & Henry 1976, Mellander 1978. Increased fluid intake is obviously not always possible and the fluid conservation by the kidneys is gradual and limited in magnitude and, further, only partly distributed to the intravascular space. Much more important, therefore, for the early plasma volume replenishment in bleeding is the redistribution of fluid from the extravascular to the intravascular compartment.

It was recognized long ago from hematocrit determinations that an entry of fluid into the circulatory system must occur in bleeding (e.g. Ebert, Stead & Gibson 1941, Kaufmann & Müller 1958), an opinion later confirmed by studies of

hemorrhagic plasma volume restitution (Lister et al 1963, Skillman, Eltringham, Goldensen & Moore 1968, Carey 1973). The observations by Chien (1958) suggested that the sympathetic nervous system, in some way or another, must be involved in such fluid transfer, because the rate of post-hemorrhagic hemodilution was found to be significantly reduced after complete sympathectomy. It seemed likely that such fluid mobilization was mainly a transcapillary event, and the mechanisms by which the sympatho-adrenal system controlled the transcapillary absorption process was demonstrated by Mellander (1960). He showed that activation of the vasoconstrictor fibres to skeletal muscle and skin caused net transcapillary fluid absorption by evoking a decrease in capillary hydrostatic pressure, in turn caused by active adrenergic resetting of the pre- to postcapillary resistance ratio. Subsequent studies by Öberg (1964) established that also reflex sympatho-adrenal activation, evoked by baroreceptor unloading, chemoreceptor activation, or by hemorrhage, caused such mobilization of extravascular fluid, mainly from skeletal muscle with its large mobile interstitial fluid reserve. Several later investigations confirmed these findings and emphasized the quantitative importance of the fluid absorption process in muscle for plasma volume restoration in hemorrhage in several species, including man (e.g. Lundgren, Lundwall & Mellander 1964, Mellander & Öberg 1967, Djojosingito, Folkow & Kovách 1968, Schwinghamer, Grega & Haddy 1970, Hall, Schwinghamer & Lalone 1976).

Compensatory fluid shifts from the extravascular to the intravascular compartment can also be evoked in hemorrhage by a glucose-osmotic mechanism via reflex neural and hormonal release of glucose from the liver leading to arterial plasma hyperosmolarity (Järhult, Lundvall, Mellander & Tibblin 1972, Järhult 1975). This mechanism seems to be of special importance in severe bleeding, although it operates gradedly in hemorrhagic hypotension (Järhult, Holmberg, Lundvall & Mellander 1976).

It has been taken more or less for granted that the adrenergic control of the pre- to postcapillary resistance ratio and of the hemorrhagic fluid absorption process in muscle is mediated by α -adrenoceptor action on the vasculature in the resistance vessels. The existence of a β -adrenergic component in the vascular resistance response in skeletal muscle to sympathetic nerve stimulation was, however, indicated by Viveros, Garlick & Renkin (1968) and this effect was shown by Lundvall & Järhult (1976) to be mainly confined to the microvessels influencing the capillary filtration coefficient and the

fluid absorption process. A microvascular dilator effect was also observed in response to noradrenaline infusion (Lundvall & Hillman 1978).

The present investigations demonstrate that the reflex sympatho-adrenal control of plasma volume and of capillary fluid transfer from skeletal muscle to blood in hemorrhage is mediated in an important way via the activation of vascular β -adrenoceptors. The experimental evidence for this hypothesis was based on quantitative observations of the hemorrhagic fluid absorption process in muscle in the absence and presence of regional β -adrenoceptor blockade (Results, section 1), on a study of the relative role of the neural and the hormonal links of the sympatho-adrenal system in this process (section 2), and on a detailed analysis of its underlying microvascular events (section 3). Further, the β -adrenoceptor specificity was determined, being of the β_2 -subtype (section 4), and the quantitative significance of β_2 -adrenergic mechanisms for overall plasma volume restitution in hemorrhage was established (section 5).

METHODS

This chapter summarizes briefly the methods used in these investigations. For detailed descriptions see original papers I-VI.

Material and anaesthesia

The experiments were performed on cats of both sexes (bwt 2.4 - 4.4 kg) anaesthetized i.v. with urethan ($100 \text{ mg} \times \text{kg bwt}^{-1}$) and α -chloralose ($50 \text{ mg} \times \text{kg bwt}^{-1}$). A recent study (Warren & Ledingham 1978) indicates that this is the anaesthesia of choice for maintenance of 'normal' cardiovascular reflex activity in hemorrhage. The animals breathed spontaneously through a tracheal cannula. Body temperature was kept at $38 \pm 0.5^\circ \text{C}$. Heparin in an effective dose was given before intravascular instrumentation.

Main surgical and experimental procedures

All observations on skeletal muscle were made on the innervated or acutely sympathectomized vascular bed of the lower hind leg muscles of the cat, separated from the body except for its main vascular connections, the popliteal artery and vein. Regional sympathectomy was accomplished either by sectioning the sciatic nerve, which contains virtually all sympathetic nerve fibres to the lower leg muscles (Cloninger & Green 1955) or by sectioning the ipsilateral lumbar sympathetic chain. The muscle preparation was placed in a fixed position inside a water-filled plethysmograph (38°C). The skin of the calf, deprived of its blood supply by dissection, was retracted to cover the muscles; it was also used to seal the proximal entrance of the plethysmograph. With this arrangement, changes of tissue volume could be followed continuously, permitting determinations of capacitance responses, net transcapillary fluid movements, and the capillary filtration coefficient (see below).

The muscle region was autoperfused from the animal in most experiments, usually under free blood flow conditions, but occasionally (IV) under constant blood flow conditions using a Harvard perfusion pump. An arterial shunt circuit was usually inserted between the ipsilateral femoral artery and the popliteal artery to permit pump perfusion and i.a. administration of drugs. In some experiments (III), the muscle region was cross-circulated via a shunt from the femoral artery of a donor animal to the popliteal artery of the recipient.

The popliteal vein was cannulated in all experiments and the outflow of blood was measured continuously with a drop recorder unit and returned to the animal via a funnel inserted in the right external jugular vein. The height of the orifice of the venous outflow tubing was adjusted so as to produce an isovolumic state in the muscle preparation in the control period (venous outflow pressure ~ 5 mmHg).

The right brachial artery was cannulated to permit rapid withdrawal of blood in the bleeding experiments which was usually accomplished within 1 min. Arterial blood pressure was continuously monitored either from this cannula or from a T-tube in the arterial shunt circuit. To be able to follow segmental vascular resistances in the muscle preparation, pressures were monitored also from arterial microvessels ('small artery pressure') and from venous microvessels ('small vein pressure'). For this purpose, minute catheters were inserted in the distal direction in the sural artery and vein after proximal ligation of the vessels according to a technique modified from that originally described by Haddy, Richards, Alden & Visscher (1954) and used in several later studies (e.g. Folkow, Sonnenschein & Wright 1971, Lundvall 1972, Lundvall & Järhult 1976, Grände 1979) in which detailed arguments were given for the validity of this approach to determine total, 'proximal arterial', 'microvascular', and 'venous' resistances (see below).

Thorough mixing of infused drugs in the blood was ensured by inserting a specially designed mixing chamber (Lundvall 1972) in the arterial shunt circuit.

Vascular reactions in the lower leg muscle region were studied in response to hemorrhage (I-III) and to stimulation of the regional sympathetic nerves and to close arterial or i.v. infusions of catecholamines (III-V).

Observations were made in the absence and presence of regional β -adrenoceptor blockade. Such blockade was in most experiments (I-IV) performed by close arterial administration of the non-selective β -adrenoceptor antagonist, propranolol, applied in a dose of $300-600 \mu\text{g} \times \text{kg muscle}^{-1}$ which proved effective by tests with isoprenaline injections. The venous outflow of blood from the muscle preparation was discarded during the propranolol infusions (~ 5 min) to avoid systemic effects. Non-specific effects of propranolol were ruled out (cf. Results). 'Selective' β_1 - and β_2 -adrenoceptor blockade was accomplished in paper V using i.v. infusions of metoprolol and compounds

H35/25 and IPS 339, respectively in the doses specified in Results (section 4) and in paper V. In paper VI the highly 'selective' β_2 -adrenoceptor antagonist, ICI 118,551, was used (section 5).

Electrical stimulation of the sympathetic nerve fibres to the muscle preparation (IV-V) was performed on the distal end of the sectioned ipsilateral lumbar sympathetic chain at the level of L 4-5 (cf. Sonnenschein & Weissman 1978). Square wave impulses (5V, 5ms, 1-10 Hz) were delivered from a Grass stimulator after atropine administration ($500 \mu\text{g} \times \text{kg bwt}^{-1}$ i.v.) in order to block the sympathetic cholinergic dilator fibres.

Selective stimulation of the preganglionic sympathetic nerves to the left adrenal medulla was performed on the sectioned splanchnic nerves (III) using the same stimulus characteristics as stated above. The right adrenal was extirpated in these experiments.

Infusions of freshly prepared solutions of catecholamines were administered at standardized rates ($0.1 \text{ ml} \times \text{min}^{-1}$ during i.a. administration and $0.3 \text{ ml} \times \text{min}^{-1}$ during i.v. administration) in graded concentrations (III-V).

Arterial plasma catecholamine concentrations were determined according to Engelman & Portnoy (1970). Plasma osmolality in arterial and in popliteal venous blood was determined by thermistor cryoscopy (Osmometer 31 LAS, Advanced Instruments, Inc.).

Plasma volume (VI) was determined with the tracer dilution technique using i.v. administered ^{125}I human serum albumin as the tracer (cf. Groom, Rowlands & Thomas 1965). To avoid blood sampling, γ -radiation was monitored in the arterial blood flowing through a defined section of a shunt circuit inserted in the left common carotid artery using an external scintillation detector connected to a scaler and linear ratemeter (for details see VI). Radioactivity was measured continuously for 50 min after each injection of the tracer to ensure complete mixing in the plasma compartment. Arterial hematocrit was measured on blood samples taken from the carotid shunt. With this technique, plasma volume was determined in the control state before bleeding and at 1 and 2 h after graded acute blood loss. Observations were made on animals with intact and systemically blocked β_2 -adrenoceptors. For this purpose, the highly 'selective' β_2 -blocking agent ICI 118,551 ($75 \mu\text{g} \times \text{kg bwt}^{-1}$ i.v.) was used (Bilski et al. 1980). The blocker was subsequently applied twice in a dose of

30 $\mu\text{g} \times \text{kg bwt}^{-1}$ to ensure a maintained effect.

Analysis of vascular functions in skeletal muscle

Vascular resistances were determined as the ratio between mean perfusion pressure and mean arterial blood flow and expressed in peripheral resistance units, PRU ($\text{mmHg} \times \text{ml}^{-1} \times \text{min} \times 100\text{g tissue}$) and thus defined as follows: Total vascular resistance = (arterial inflow pressure - venous outflow pressure)/flow; 'proximal arterial resistance' = (arterial inflow pressure - small artery pressure)/flow; 'microvascular resistance' = (small artery pressure - small vein pressure)/flow; 'large vein resistance' = (small vein pressure - venous outflow pressure)/flow.

Precapillary 'sphincter' activity, determining the number of patent capillaries and, hence, the size of the functional capillary surface area available for transcapillary fluid exchange, was estimated by measurements of the capillary filtration coefficient (CFC). This method has been established to provide valid information about alterations of precapillary 'sphincter' tone, provided the specific capillary fluid permeability is not altered in response to the experimental intervention (Cobbold, Folkow, Kjellmer & Mellander 1963, Folkow & Mellander 1970). In the present study this requirement seemed to be fulfilled, as indicated by test experiments showing that even pronounced adrenergic activation by high concentrations of adrenaline, or intense sympathetic nerve stimulation, did not change the CFC in situations where precapillary 'sphincter' tone prior to these stimuli was completely abolished by papaverine infusion. CFC was calculated from the volumetrically determined rate of net transcapillary fluid filtration caused by raising venous outflow pressure 5 mmHg, assuming 80% of this pressure rise to be transmitted to the capillary level. It should be noted that even if this assumption is not entirely valid since the resistance ratio varied in the present experiments the resulting error in the calculated CFC value will be small (see Cobbold et al. 1963).

Data on capacitance responses and net transcapillary fluid movements were derived from observed changes of tissue volume according to the principle described by Mellander (1960). Adrenergically induced abrupt changes of tissue volume, coordinated in time with changes of vascular resistance, are considered to reflect the active and passive responses of the capacitance vessels, whereas the subsequent gradual change of tissue volume, occurring in

face of a maintained steady state resistance response, is considered to represent net transfer of fluid across the capillaries (Mellander 1960, Öberg 1967). This interpretation of the volume curve has been validated in several studies by the simultaneous use of the volumetric technique and a radioactive tracer method which permits selective determination of the regional blood volume change, i.e. capacitance response (e.g. Åblad & Mellander 1963, Kjellmer 1965). This dual approach was used in a few test experiments of this study, validating, in principle, the interpretations of the tissue volume curves made below.

Estimation of glucose-osmotic fluid absorption

As described in Introduction and in Results, the compensatory fluid absorption from the extravascular space of skeletal muscle into the intravascular compartment during hemorrhage is partly due to a decrease in capillary hydrostatic pressure and partly the result of a glucose-osmotic process. Estimation of the glucose-osmotic component of the observed net transcapillary fluid absorption during bleeding was partly based on a theoretical analysis of this process (Lundvall 1972). Using a simple capillary model he showed mathematically that the osmotic fluid flux in skeletal muscle varied directly with the product: Blood flow x the arterio-venous osmolar difference in the capillary. This relation was later confirmed in principle by Salathe & An (1976). The blood flow dependency is related to the effect of flow on the transcapillary osmolar gradient. High blood flow rates tend to preserve the gradient, whereas low flow rates facilitate rapid transcapillary osmotic equilibration due to transcapillary solute diffusion and osmotic fluid (water) flux. Both the osmolar difference and the blood flow can be measured experimentally. In vivo studies confirmed the theoretical analysis and established the following relation between hyperglycemic osmotic fluid flux, blood flow, and (A-V) osmolar difference (cf. paper I):

$$\text{Fluid flux rate} = \text{A-V osmolar difference} \times \text{blood flow} \times 0.175 \times 10^{-2} \quad (1)$$

where the fluid flux rate is expressed in $\text{ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$, and the osmolar difference in $\text{mOsm} \times \text{kg H}_2\text{O}^{-1}$. The constant (0.175×10^{-2}) is determined, among other things, by the osmotic reflexion coefficient for glucose. Note, however, that the reflexion coefficient per se, which can be estimated to be

in the range of 0.1 - 0.3 (see Perl 1971, Lundvall 1972), need not be known for calculation of the osmotic fluid flux.

Statistics

Spread of data is given as standard error of the mean (SE). Student's t-test was used for evaluation of statistical significance (* denotes $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). The non-parametric Wilcoxon's rank comparison test was applied, in addition, in some of the studies (II, V).

RESULTS AND COMMENTS

1. BETA-ADRENERGIC CONTROL OF TRANSCAPILLARY FLUID ABSORPTION IN SKELETAL MUSCLE IN HEMORRHAGE

This section summarizes results (papers I-II) which provide evidence for the hypothesis that the compensatory plasma volume control in hemorrhage, related to reflex transcapillary fluid absorption from skeletal muscle, is mediated to a major extent by vascular β -adrenergic mechanisms. The analysis was based on comparative observations of the rates of fluid absorption, and of associated circulatory events, in bleeding occurring in the absence and presence of regional β -adrenoceptor blockade in skeletal muscle.

Principal pattern of response to standardized hemorrhage

Fig. 1 (previously unpublished) illustrates typical patterns of vascular response to hemorrhage in the cat's lower leg muscles with 'intact' (panel A) and regionally blocked (panel B) β -adrenoceptors. Effective β -adrenoceptor blockade was instituted by close arterial infusion of propranolol to the muscle preparation prior to hemorrhage. Bleeding in this and all other experiments was accomplished by standardized rapid withdrawal of blood (Fig. 1, horizontal bars), in this case of $10 \text{ ml} \times \text{kg bwt}^{-1}$ corresponding to about 20% of the cat's total blood volume (cf. section 5).

In the absence of β -adrenoceptor blockade, hemorrhage caused a rapid decrease in arterial blood pressure stabilizing around 105 mmHg and an increase in vascular resistance by about 30%. Tissue volume, being approximately constant in the control period (isovolumic state), showed an initial rapid decrease coordinated in time with the development of the resistance response (see vertical broken line) which reflects the mobilization of blood from the region, i.e. the capacitance response (see Methods). The subsequent slower decline of tissue volume represents the net transcapillary fluid absorption from tissue to blood, which in the steady state stabilized at a relatively constant rate of $0.07 \text{ ml} \times \text{min}^{-1} \times 100\text{g tissue}^{-1}$ (see dotted lines beneath the original tracing which neglects the volume effect of the superimposed CFC determination). Effects on CFC will be described in section 3. About 10 min after the bleeding, the shed blood volume was reinfused, which led to gradual circulatory recovery.

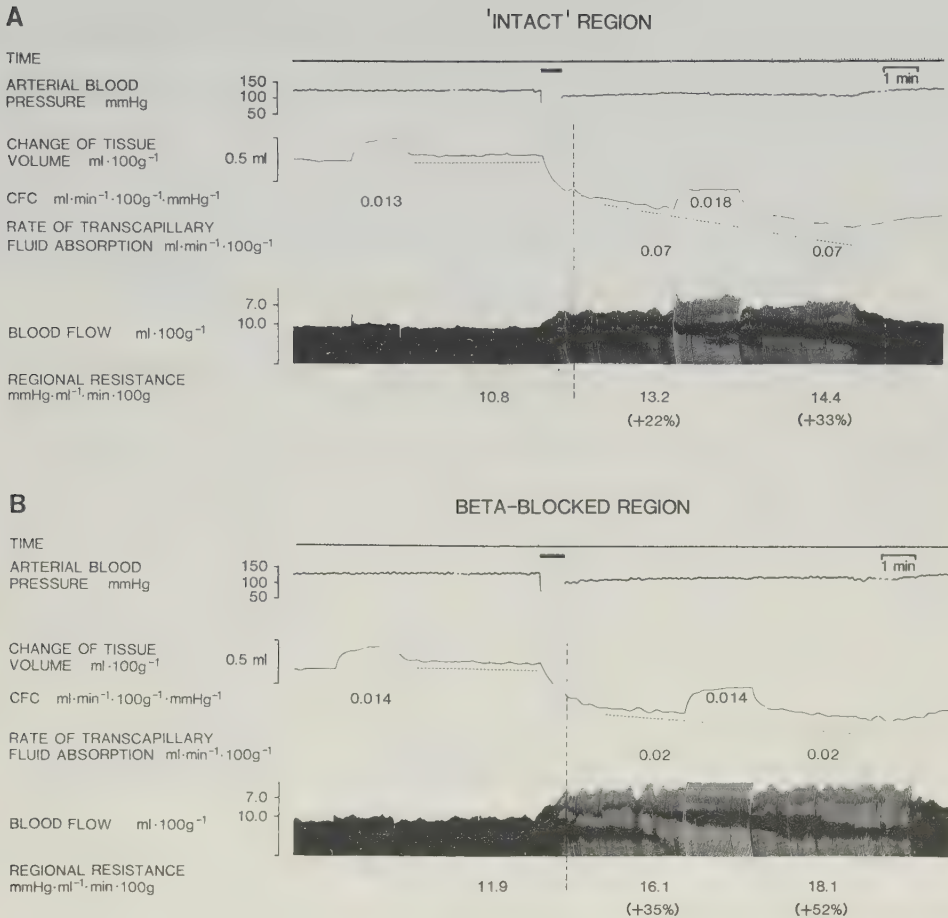


Fig. 1. Original tracings illustrating the patterns of vascular response to acute hemorrhage (20% of total blood volume, see horizontal bars) in the lower leg muscles of the cat before and after regional β -adrenoceptor blockade (propranolol). The capacitance response, the transcapillary fluid absorption rate, and the capillary filtration coefficient were determined from observed changes of tissue volume. The termination of the capacitance response is indicated by the vertical broken line and the rate of fluid absorption by the dotted lines. The more than three times faster fluid absorption rate on 'intact' than β -blocked muscle preparation indicates an important β -adrenergic control of transcapillary fluid absorption in bleeding.

Panel B shows the corresponding responses to equivalent blood loss in the presence of effective regional β -adrenoceptor blockade in the same animal. The effects on blood pressure and on the capacitance vessels were similar to those shown in panel A, but the resistance response after β -blockade was clearly

reinforced (resistance increase about 50%) and the steady state transcapillary fluid absorption rate was clearly diminished (to about $0.02 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$). The rate of transcapillary fluid absorption was thus more than three times faster in the muscle region with intact than with blocked β -adrenoceptors despite the fact that regional resistance was less augmented in the former than the latter situation.

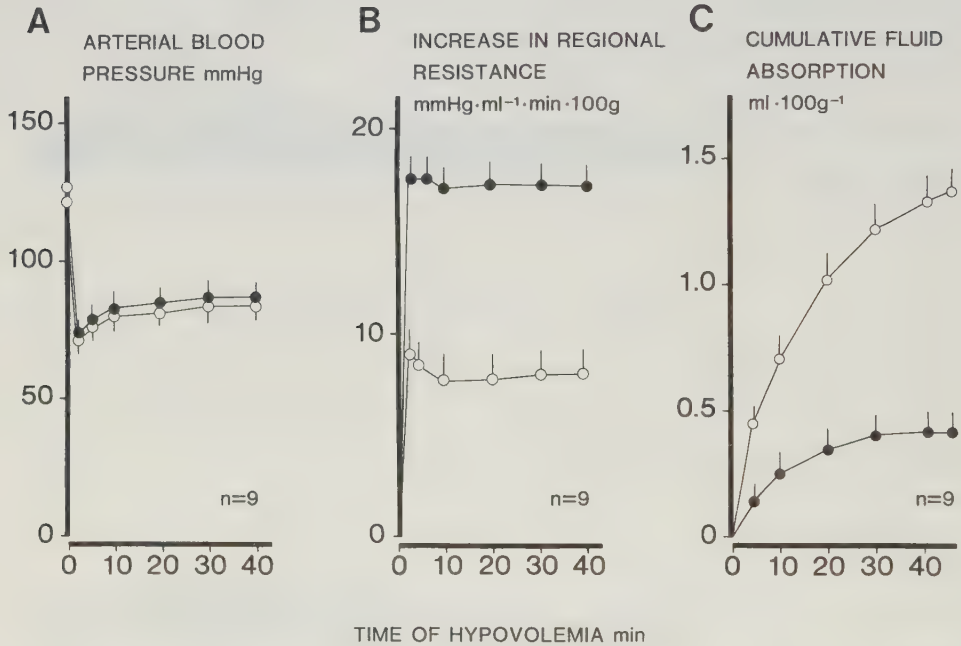


Fig. 2. Collected data from 18 cats on decrease in arterial blood pressure, increase in vascular resistance, and cumulative transcapillary fluid absorption in the muscle preparation with intact (open circles) and blocked (closed circles) β -adrenoceptors during a 45 min observation period after acute 20% reduction of the total blood volume.

Fig. 2 shows collected data from 18 cats on arterial blood pressure, increase in vascular resistance, and cumulative transcapillary fluid absorption in the muscle preparation with intact (open circles) and blocked (closed circles) β -adrenoceptors during a 45 min observation period after standardized blood loss (20% of total blood volume). The blood pressure decline was not different in these two series of experiments. However, the vascular resistance increase in the muscle preparation was some 100% greater in the presence than in the absence of regional β -adrenoceptor blockade. With intact β -adrenoceptors, the fluid absorption process was marked and maintained throughout the observation period, though its rate was declining gradually with time. The total volume of

extravascular fluid transferred into the blood stream in 45 min averaged $1.4 \text{ ml} \times 100\text{g muscle}^{-1}$. After β -blockade, the fluid absorption process was very much attenuated and it virtually ceased half an hour after the initial blood loss. The total absorbed fluid volume in 45 min was $0.4 \text{ ml} \times 100\text{g}^{-1}$, i.e. only about 30% of that occurring in the muscle with intact β -adrenoceptors.

These data strongly indicate that the transcapillary fluid absorption process in skeletal muscle after acute blood loss is mainly, but not entirely, governed by regional vascular β -adrenoceptor mechanisms, the nature of which will be analysed in greater detail in sections 2-4 of Results.

Pattern of response to graded hemorrhage

If the β -adrenergic control of transcapillary fluid absorption from skeletal muscle is a component in the overall homeostatic volume regulation in bleeding, the rate of absorption should be expected to be strictly graded in response to the magnitude of the shed blood volume. This problem was approached by observing the rates of fluid absorption and the vascular resistance responses to rapid withdrawal of 10, 20, and 40% of the cat's total blood volume in muscle preparations with intact or blocked β -adrenoceptors. For the sake of reasonable standardization of the experimental conditions, this analysis was limited to short-term observations (10 min) after bleeding. In these graded hemorrhage experiments, arterial blood pressure in the steady state averaged 109 ± 2 , 88 ± 4 , and 70 ± 6 mmHg in the animals with 'intact' preparation ($n=7$) and the values were not significantly different after regional β -blockade ($n=7$). Fig. 3 shows the vascular resistance responses and the rates of fluid absorption in these experiments determined 8-10 min after the start of the bleeding. It can be seen from panel A that the increases in vascular resistance were graded in relation to the severity of the bleeding both on the preparation with intact and blocked β -adrenoceptors. However, the resistance increase was much less pronounced with intact than blocked β -adrenoceptors, apparently due to a β -adrenergic dilator interaction with a concomitant, mainly α -adrenergic, constriction of the resistance vessels, the latter component being fully unmasked by β -blockade. The β -adrenergic attenuation of the resistance response (represented by the difference between the two resistance curves) was greater the larger the shed blood volume. The data in panel B show that also the rates of transcapillary fluid absorption in muscle were clearly graded in relation to the magnitude of the blood loss both on preparations with intact and blocked β -adrenoceptors, but the absorption

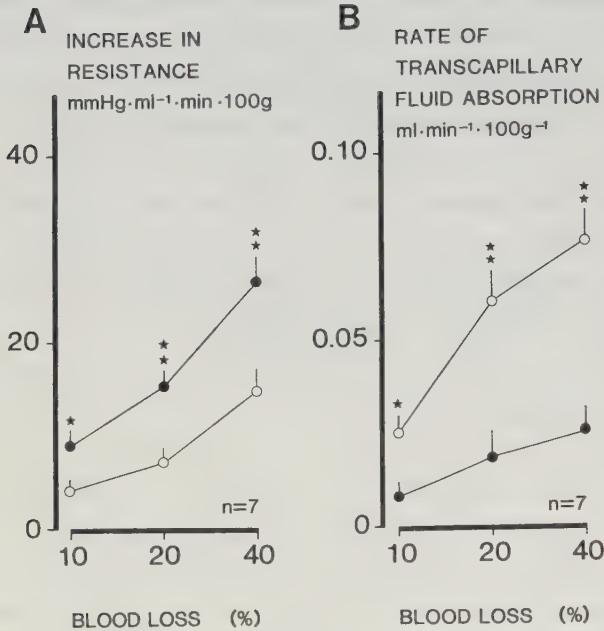


Fig. 3. Collected data (n=7) illustrating the increases in vascular resistance, and the rates of transcapillary fluid absorption in the muscle preparation with intact (open circles) and blocked (closed circles) β -adrenoceptors observed in the steady state 8-10 min after rapid withdrawal of 10, 20, and 40% of the cat's total blood volume.

rates were much faster in the former at any level of hypovolemia. The β -adrenergic contribution to this absorption process (represented roughly by the difference between the two curves for fluid absorption in panel B, see Comments) was directly related to the severity of bleeding thus indicating that the β -adrenergic control of the fluid absorption process operates gradedly over the investigated range of hypovolemia.

COMMENTS

These experiments indicate that the transcapillary fluid absorption process in skeletal muscle, evoked in hemorrhage, is governed to a significant extent by a reflex β -adrenergic control mechanism, operating in concert with the previously established α -adrenergic and glucose-osmotic regulatory mechanisms mentioned in Introduction. The interpretation of the results and the evaluation of the extent to which these different mechanisms contributed to the described fluid absorption require some methodological and general considerations.

The fluid absorption process in muscle was here studied with the volumetric

technique originally described by Mellander (1960). From quite extensive critical examinations of this technique (see Methods) it can be considered to permit reliable estimates of net transcapillary fluid movement in muscle. Important in this context is that the vascular capacitance response can be separated from the transcapillary fluid absorption process in hemorrhage. This was facilitated in the present investigation by the bleeding procedure employed. The animals were exposed to a rapid standardized blood loss which implies that both the resistance and the capacitance responses are fully developed within short period of time, usually in less than 1 min, after the blood withdrawal, as verified also by special tests with the radioactive tracer technique mentioned in Methods. Further it should be stressed that fluid transport from the muscle preparation via the lymphatic system was prevented in these experiments by ligation of the regional lymph vessels and that, therefore, the described fluid absorption is a true capillary event.

The present experimental model of bleeding by which a sudden fixed blood volume was withdrawn simulates a common type of acute hemorrhage in patients in which effective hemostasis is successfully instituted shortly after the blood loss. This experimental approach has been used in several previous studies (e.g. Swan 1965). Such a well-defined exsanguination imposes on the experimental animal a given acute circulatory stress which, via the various cardiovascular receptors, causes prompt reflex activation of the sympatho-adrenal system (e.g. Chien 1967). This, in turn, evokes compensatory adjustments from which the animal can benefit with gradual more or less complete restoration of cardiovascular homeostasis as a result. This experimental bleeding technique therefore seems to offer quite optimal conditions for a detailed analysis of the mechanisms behind transcapillary fluid mobilization from skeletal muscle.

In many previous investigations another experimental bleeding model has been employed by which a fixed and maintained arterial hypotension level is achieved with the use of a pressure bottle connected to the arterial system of the animal (Wiggers & Werle 1942, Lamson & DeTurk 1945). This experimental bleeding technique implies that the animal cannot benefit much from the physiological compensatory adjustments brought into play after bleeding. Hence, it causes an artificially prolonged and excessive circulatory stress to the animal which is more directly related to the arterial hypotension than to the hypovolemia per se. However, the loss of blood volume is the primary disturbance in hemorrhage and the decrease in arterial pressure only one of

its secondary consequences. Such considerations may further justify the use of the acute, fixed volume, bleeding technique for the present analyses.

With the present experimental design it is believed that, for a given acute blood loss, the overall reflex sympatho-adrenal activation was closely similar in animals with intact and regionally blocked β -adrenoceptors. This assumption was supported by the finding that for a given experimental hypovolemia the arterial hypotension level was almost identical in these two groups of animals (e.g. Fig. 2, panel A). It should be recalled in this context that the β -blockade was strictly confined to the small lower leg muscle preparation, comprising only some 2% of the total body weight, and that there was no systemic effect of propranolol. Yet, the local propranolol treatment would eliminate the possible presynaptic β -adrenergic enhancement of transmitter release at the sympathetic nerve endings (Westfall 1977, Langer 1981) in the muscle preparation in hemorrhage. Such a presynaptic effect is, however, reported to be quite small in blood vessels during nerve stimulation, at least with regard to the resulting effector response (Dahlöf et al. 1975, Stjärne & Brundin 1975, Dahlöf 1981). During reflex sympatho-adrenal activation, blood-borne adrenaline might be an effective endogenous agonist for such presynaptic β -adrenoceptors (Stjärne & Brundin 1975, Dahlöf 1981) thereby reinforcing to some extent the neurotransmitter release in the absence of β -blockade. Non-specific, e.g. local anaesthetic, effects of propranolol in the applied dose can be considered to be insignificant, as indicated by test experiments with its dextro-isomer, being practically devoid of β -blocking properties (e.g. Barrett & Cullum 1968, Lundvall & Järhult 1976). A vivid reflex sympatho-adrenal activation of the vasculature in the β -blocked muscle region was further evidenced by the strong vascular resistance response to bleeding. The finding that the reflex resistance increase in fact was greater in the β -blocked than in the intact region to a given blood loss (Fig. 2, panel B) most likely can be ascribed to the unmasked α -adrenergic influence on the regional resistance vessels accomplished by β -blockade, the latter thus eliminating the β -adrenergic dilator interaction present in the vascular bed with intact β -adrenoceptors.

It follows from these considerations that the reflex sympatho-adrenal outflow to the muscle preparation in the absence and presence of regional β -adrenergic blockade can be considered to be approximately equal in matched bleeding experiments. The present comparative results therefore indicate that the β -adrenergic mechanism on the intact preparation exerts a significant

interaction with concomitant constrictor mechanisms on the vascular resistance function, and a pronounced facilitating effect on the transcapillary fluid absorption process in skeletal muscle during hemorrhage. Note, however, that the constrictor mechanism (which is mainly α -adrenergic in nature, as also evidenced by observations after regional α -adrenergic blockade) overrides the β -adrenergic influence on the overall resistance function in muscle during hemorrhage. It is further noteworthy that the α -adrenergic influence seems to entirely dominate the control of the capacitance vessels being little, if at all, affected by β -adrenergic mechanisms (Fig. 1).

A main new finding in this series of experiments was that β -adrenergic mechanisms contribute importantly to the transcapillary fluid absorption from muscle in hemorrhage, a process which by previous studies, as mentioned, was considered to be governed mainly by α -adrenergic actions on the vasculature and by a glucose-osmotic mechanism. No doubt, the latter two mechanisms were responsible to some extent for the fluid absorption described in the present experiments, and most likely fully explain the absorption remaining in these experiments after regional β -adrenergic blockade. The question arises whether the difference between the fluid absorption observed in the muscle with intact and blocked β -adrenoceptors (Fig. 1 and 2) can be taken as an approximately correct measure of the absorption fraction mediated by the β -adrenergic mechanism per se. This assumption presupposes that the α -adrenergic and glucose-osmotic mechanisms for fluid absorption were about equally effective before and after β -blockade.

The α -adrenergic constrictor influence on the resistance vessels was unmasked and, hence, reinforced on the muscle preparation with blocked β -adrenoceptors and, therefore, would be expected to contribute more effectively, if anything, to fluid absorption in the presence than in the absence of β -blockade, hence possibly leading to some underestimation of the role of the β -adrenergic absorption effect. The glucose-osmotic contribution to this process was subjected to special analysis in these experiments, which deserves some summarizing comments.

Hemorrhage is known to be associated with hyperglycemia evoked by reflex sympathetic and hormonal influences on glucose release from the liver. This raises the osmolality of the arterial blood and establishes, as one consequence, a transcapillary glucose-osmotic driving force for fluid absorption from tissue to blood, especially in skeletal muscle (Järhult 1975).

If the sympatho-adrenal activation is very strong and long maintained, as it is experimentally when the mentioned constant arterial hypotension bleeding model of Wiggers is employed, the arterial glucose hyperosmolality can be pronounced, in severe bleeding raising arterial osmolality by more than 20 mOsm $\times$ kg H_2O^{-1} above isotonicity. It then contributes most significantly to the transcapillary fluid absorption process in muscle (Järhult 1975). In the present experiments, however, in which hemorrhage was accomplished by rapid withdrawal of a standardized volume of blood, the arterial glucose hyperosmolality was much more moderate, seldom exceeding 5 mOsm $\times$ kg H_2O^{-1} , as found by routine testing in all experiments. Further, the arterial glucose hyperosmolality, and the calculated glucose-osmotic transcapillary driving force in muscle (see Methods), was found not to be altered by regional β -adrenoceptor blockade. This implies that the fluid absorption caused by this factor should be present also after β -blockade and, hence, be largely included in the residual absorption described after such treatment. However, the glucose-osmotic absorption process is known to be blood flow dependent (Lundvall 1972, Salathe & An 1976), roughly according to equation (1) in Methods, which was employed to take into consideration differences in blood flow in the intact and the β -blocked preparations. Such estimations led to the conclusion that the glucose-osmotic absorption was not much different in the intact and the β -blocked preparation. In the former, it comprised at most some 20% of the total fluid absorption both at short-term (10 min) graded bleedings (Fig. 3) and at prolonged (45 min) bleeding (Fig. 2).

It could be argued that the observed fluid absorption during bleeding to some extent could be an effect of the arterial blood pressure decline, in turn passively lowering capillary hydrostatic pressure. This does not seem to be the case. Capillary hydrostatic pressure in skeletal muscle has been shown to remain roughly constant by active autoregulation over the entire range of arterial pressure from 170 down to 30 mmHg under normal resting conditions (Järhult & Mellander 1974, cf. Johnson 1980). Specific tests in the present series of experiments (Lundvall & Hillman, to be published) showed that this autoregulatory capacity was well maintained also during bleeding. Passive effects of changed venous pressure on the absorption process were eliminated in these experiments by keeping venous outflow pressure constant. It follows that the hemorrhagic transcapillary fluid absorption in muscle related to β -adrenergic mechanisms was not due to such passive effects of changed arterial or venous pressure, but caused by active vascular smooth muscle adjustments in the microcirculation, as evidenced in section 3 of Results.

These considerations seem to warrant the conclusion that the difference in the transcapillary absorption rates observed in the muscle region with intact and blocked β -adrenoceptors adequately reflects the contribution to this process effected by β -adrenergic control.

In an attempt to visualize the functional significance of the described fluid absorption process for the compensatory plasma volume restoration in bleeding, the absorption data for the lower leg muscle preparation will be extrapolated to apply to the whole skeletal muscle mass in the cat. The wet weight of the total muscle mass was found to average 32% of the cat's body weight, as determined in 5 animals after dissection, i.e. about 1 kg in a medium-sized 3 kg cat. The cumulative fluid absorption in the muscle preparation was $1.4 \text{ ml} \times 100 \text{g tissue}^{-1}$ (Fig. 2) or, by extrapolation, 14 ml in the whole muscle mass, in a 45 min period after initial withdrawal of 20% of the cat's total blood volume, (i.e. a total plasma volume loss of about 20 ml, arterial hematocrit being 34%). This implies a restoration of some 2/3 of the total initial plasma volume deficit by the fluid absorption process from muscle in this period of time, a major part of which can be attributed to the β -adrenergic control (Fig. 2). Such rough a deduction suggests an important role of this absorption process in the compensatory plasma volume restoration in hemorrhage, a problem which will be more specifically approached in section 5 by direct determination of plasma volume restitution in bleeding.

2. ROLE OF HORMONAL AND NEURAL MECHANISMS IN THE BETA-ADRENERGIC CONTROL OF THE FLUID ABSORPTION PROCESS IN HEMORRHAGE

The results in the previous section led to the conclusion that the transcapillary fluid absorption from muscle in response to acute hypovolemia to a major extent was mediated by reflex β -adrenergic mechanisms and that such absorption was maintained for considerable length of time (Fig. 2). It has been established previously that direct stimulation of the sympathetic vasoconstrictor fibres to skeletal muscle leads to pronounced transcapillary fluid absorption, a phenomenon which, however, is relatively transient in nature ending within some 5 min despite continued stimulation and vasoconstriction (Mellander 1960, Lundvall & Järhult 1976). It is therefore likely that the described adrenergic prolongation of the absorption process in bleeding is mainly mediated by the hormonal link of the sympatho-adrenal system and, then, especially by adrenaline. In an attempt to reveal the relative role of the sympathetic neural and hormonal influences on the transcapillary fluid absorption in response to standardized blood loss, the process was compared quantitatively 1) on animals with intact sympatho-adrenal system, 2) under conditions of selective reflex activation of the regional vasomotor fibres (cross-circulation experiments), and 3) under selective humoral influence on the vasculature (after regional sympathectomy). To determine the β -adrenergic contribution to the absorption responses, such analyses were performed before and after regional β -adrenoceptor blockade.

Fig. 4 illustrates the principal patterns of fluid absorption observed in response to acute hemorrhage (20% of the cat's total blood volume) under these three experimental conditions. The data were obtained by recording change of tissue volume and refer to original tracings presented in paper III. To facilitate the comparison, the original volume curves were superimposed and reconstructed to fit a common volume scale. CFC determinations made in the original experiments were omitted in this figure and replaced by extrapolated broken lines.

It can be seen that the acute blood loss led to a decrease in tissue volume in all three experiments, initially caused by a capacitance response which was completed at the time indicated by the arrow below the volume curves. This response was coordinated with the development of the resistance response (see original tracings in Figs. 1-3, paper III). The transcapillary fluid absorption rate in the muscle preparation is reflected by the tissue volume

decline occurring after the capacitance response.

It can be seen that the reflex neural mechanism, in selective operation in the cross-circulation experiment, caused a pronounced capacitance response and fast rate fluid absorption which, however, ceased within some 5 min of reflex activation. The humoral mechanism, revealed after sympathectomy, in contrast caused virtually no capacitance response, but effective net absorption over an extended period of time. By additive effects, these two mechanisms fully explain the capacitance response and the high fluid absorption rate observed in the animal with intact sympatho-adrenal system (Fig. 4, curve 1). It is concluded that the sympathetic neural mechanism dominates the control of the capacitance function, but only transiently influences the fluid absorption process. The humoral mechanism, on the other hand, seems to be responsible for the maintenance of the transcapillary fluid absorption in bleeding.

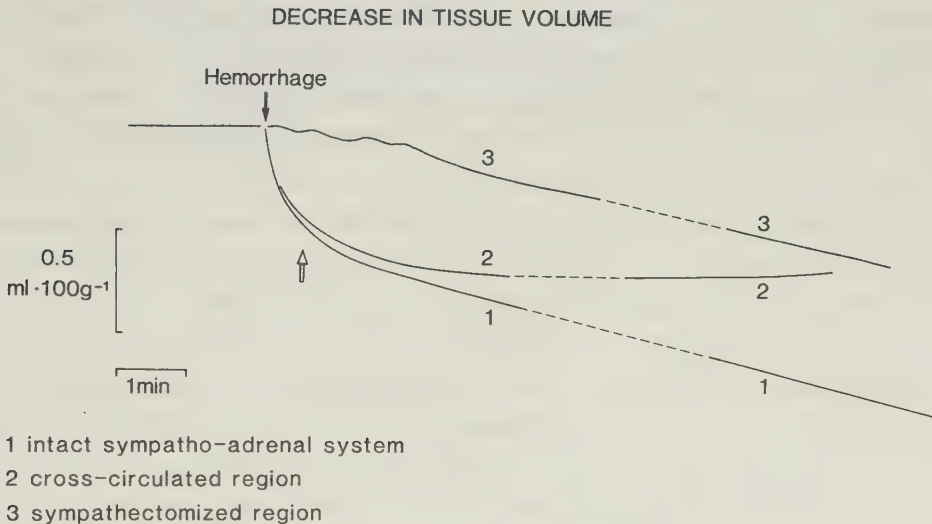


Fig. 4. Principal patterns of tissue volume change, reflecting capacitance responses and transcapillary fluid absorption rates in the autoperfused innervated (1), autoperfused sympathectomized (3) and cross-circulated innervated (2) vascular bed of skeletal muscle evoked by an acute 20% reduction of the blood volume. The termination of the capacitance responses is indicated by the arrow below the volume curves. For further description see text.

The humoral component in the hemorrhagic fluid absorption process might be ascribed to the action of catecholamines released from the adrenal medulla and

to other blood-borne factors, such as hyperosmolarity, systemic 'overflow' of noradrenaline from sympathetic nerve endings or other humoral vasoactive agents. To evaluate the role of the adrenal catecholamines per se, the rate of fluid absorption in the steady state in response to standardized bleeding was determined in the sympathectomized muscle region before and after bilateral adrenalectomy (Table 2, III). The results demonstrated that the adrenal catecholamines were responsible for the major part (about two thirds) of the fluid absorption caused by blood-borne factors, the remainder being mainly evoked by the glucose-osmotic mechanism.

The contribution of β -adrenergic control mechanisms to the hemorrhagic fluid absorption was estimated by comparative studies before and after regional β -blockade (Table 3, III). The following data provide a brief summary of these results. In the phase after hemorrhage when the absorption process could be considered to be almost entirely controlled by humoral mechanisms, i.e. >5 min after the acute blood loss (20%), the rate of transcapillary fluid absorption averaged $0.069 \pm 0.010 \text{ ml} \times \text{min}^{-1} \times 100\text{g muscle}^{-1}$ with intact β -adrenoceptors and, as expected, it was not significantly different after regional sympathectomy. After β -adrenoceptor blockade, the absorption rate decreased by about 60%, indicating an important β -adrenergic component in the response. The neurogenically controlled fluid absorption (occurring in the early phase of hypovolemia) also seemed to be mediated, in part, by β -adrenergic mechanisms, as evidenced by the finding that the cumulative nerve induced fluid absorption (determined in cross-circulation experiments) of $0.35 \pm 0.03 \text{ ml} \times 100\text{g}^{-1}$ was reduced by 45% after β -blockade.

The adrenaline and noradrenaline concentrations in arterial plasma in the steady state 10 min after a 20% blood loss rose on the average by about 20 and 40 nM, respectively (II). In a separate series of experiments, the fluid absorption in the sympathectomized muscle preparation was observed in response to i.v. infusions of these two catecholamines in doses raising the arterial plasma concentrations to the same levels as in hemorrhage (III). Such infusions led to a net transcapillary fluid absorption response which was well maintained during the infusion period (tested for up to 20 min, see for instance Fig. 5, III), provided there was a simultaneous net constrictor response of the resistance vessels. Similar well maintained absorption responses were evoked by longlasting (up to 45 min) stimulation of the preganglionic splanchnic nerves supplying the adrenal medulla (III).

COMMENTS

It can be concluded from these experiments that the transcapillary fluid absorption process in skeletal muscle in hemorrhage is governed by the neural as well as the hormonal links of the sympatho-adrenal system. The direct neural control of the process is prompt in onset and leads to fast rate absorption in the initial phase after bleeding, but the effect seems to be transient in nature. Despite this, the neural absorption was estimated to be responsible for rapid replacement of some 15-20% of the shed plasma volume in the present experiments, a figure derived by extrapolation of the data to the whole muscle mass in the cat. The adrenergic hormonal control is somewhat slower in onset and may cause less pronounced fluid absorption per unit time, but the effect is maintained for considerable length of time and, therefore, becomes especially important for the long-term cumulative fluid gain into the circulatory system in bleeding. The hormonal link no doubt also is of great importance for the control of the resistance function in muscle during hemorrhage (cf. Bond, Manley & Green 1967, Hall et al. 1976).

The finding that the sympatho-adrenal regulation of the fluid absorption process in muscle is organized in such a way that the neural link causes only transient effects whereas the hormonal link causes prolonged effects may have important implications when considering the overall control of cardiovascular homeostasis (see General Discussion).

With regard to the β -adrenergic contribution to the absorption process in bleeding it should be stressed that β -adrenergic mechanisms were involved both in the neural and the hormonal response. As expected, the β -adrenergic component was most prominent in the hormonal control. This effect is probably mainly linked to the action of adrenaline, but also noradrenaline can contribute to fluid absorption via β -adrenergic mechanisms. The site of action of such β -adrenergic influence in the microcirculation will be analysed in the next section of Results.

3. BETA-ADRENERGIC CONTROL OF FUNCTIONAL CAPILLARY SURFACE AREA AND CAPILLARY HYDROSTATIC PRESSURE IN SKELETAL MUSCLE IN HEMORRHAGE

This section summarizes results of experiments (I-IV) aimed at revealing vascular effects in the microcirculation which can explain the described β -adrenergic control of transcapillary fluid absorption in bleeding. Among the several factors contributing to the normal Starling fluid equilibrium across the capillaries there are, in principle, only two which can be directly controlled by change of vascular smooth muscle tone, viz. the capillary filtration coefficient, CFC, and the capillary hydrostatic pressure, P_c (cf. Mellander 1978). CFC is influenced by the activity in the precapillary 'sphincters', via their control of the size of the functional capillary surface area. At constant arterial and venous pressures, P_c is actively controlled by resettings of the pre- to post-capillary resistance ratio, R_a/R_v (see Mellander & Johansson, 1968). The results to be described indicate that the β -adrenergic control of fluid absorption in bleeding is effected by adjustments of both CFC and R_a/R_v .

Beta-adrenergic control of functional capillary surface area

Fig. 5, panel A, shows CFC values in the muscle preparation with intact and blocked β -adrenoceptors during graded short-term (10 min) hemorrhage. In the control period prior to bleeding, CFC averaged $0.014 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1} \times \text{mmHg}^{-1}$. It can be seen from the figure that with intact β -adrenoceptors, hemorrhage caused a marked increase in CFC which was quantitatively related to the severity of the bleeding. After regional β -blockade, CFC was much less augmented. This difference indicates the existence of a β -adrenergic inhibitory control of the precapillary 'sphincters' and a consequent enlargement of the functional capillary surface area available for fluid exchange. Without such an influence, the functional capillary surface (CFC) was only slightly increased, an effect most likely due to a metabolic influence caused by the concomitant blood flow reduction, (cf. Mellander & Lewis 1963, Cobbald et al. 1963). The β -adrenergic relaxation of the precapillary 'sphincters' was sustained in bleeding, as indicated by the CFC data in panel B, referring to observations during 40 min after a 20 % acute blood volume reduction.

From corresponding observations of CFC on the sympathectomized and cross-circulated muscle preparations after standardized blood loss (III) the

conclusion was drawn that the β -adrenergic control of the precapillary 'sphincters' was mediated both via the neural and hormonal links of the sympatho-adrenal system.

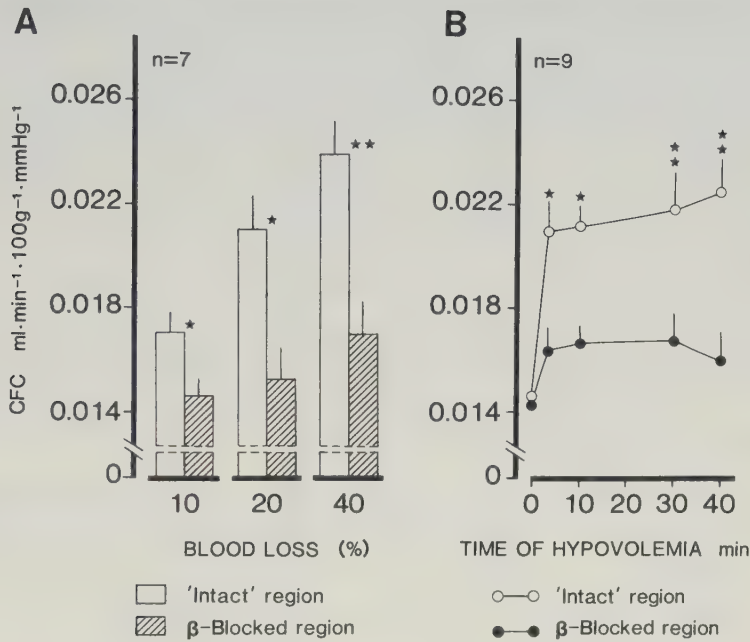


Fig. 5. Data on CFC in skeletal muscle with intact and blocked β -adrenoceptors during hemorrhage. Panel A shows CFC values in the steady state during short term (10 min) graded hemorrhage: 10, 20, and 40% reductions of total blood volume. Panel B shows CFC values determined during a 40 min period after an acute 20% blood loss. The control CFC value before bleeding averaged $0.014 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1} \times \text{mmHg}^{-1}$. CFC was significantly greater on the 'intact' than β -blocked region, indicating a β -adrenergic enlarging effect on the functional capillary surface area in hemorrhage.

Further support for such a dual control was obtained from analyses of CFC during close arterial catecholamine infusions and during stimulation of the regional sympathetic nerves to the muscle region with intact and blocked β -adrenoceptors (Fig. 6). The CFC effects are plotted versus the simultaneously evoked increase in regional vascular resistance. The infused amounts of the catecholamines were calculated to produce arterial plasma concentrations in the steady state ranging from very low values up to about 450 nM, which mimics concentrations reported in the literature during hemorrhage (e.g. Watts 1965, Callingham 1975, Andersson et al. 1982). Sympathetic stimulation was applied in the range from 1-10 Hz. The CFC data indicate that adrenaline, even in low concentrations, exerts a powerful β -adrenergic relaxing effect on the precapillary 'sphincters' in the preparation with intact β -adrenoceptors. It

apparently also exerts a pronounced β -adrenergic inhibitory effect on the resistance vessels, counteracting the α -adrenergic constriction, since the resistance increase with adrenaline was small on the intact, but marked on the β -blocked, preparation. Blood-borne noradrenaline and the sympathetic nerve fibres exert similar β -adrenergic effects, although for a given resistance increase they were less pronounced than with adrenaline.

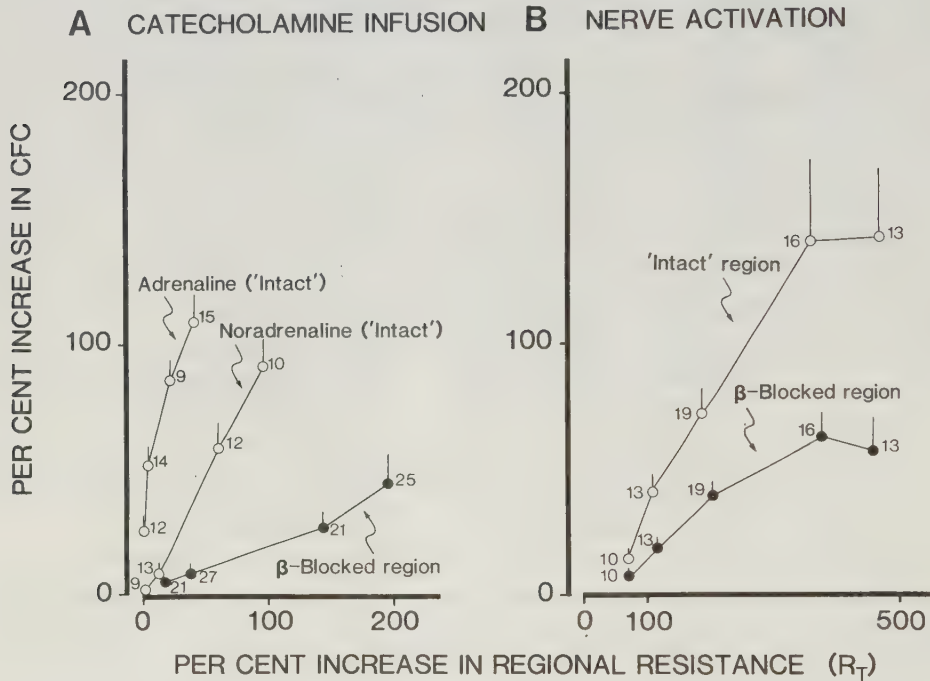


Fig. 6. Simultaneous CFC and vascular resistance responses in the muscle preparation with intact (open circles) and blocked (closed circles) β -adrenoceptors evoked by graded close arterial infusions of noradrenaline and adrenaline (panel A) and by graded sympathetic nerve stimulation (panel B). The response to the two catecholamines on the β -blocked preparation are lumped together in Panel A. The numerals in the diagrams denote the number of observations.

It should be stressed that the full range of neural adrenergic activation accomplished in these experiments was hardly encountered in the present hemorrhage experiments, since regional resistance on the β -blocked muscle preparation with unmasked α -adrenergic influence seldom exceeded the control value by more than 150%. The relaxation of the precapillary sphincters in the β -blocked region, indicated by the gradual rise of CFC with increasing vascular resistance (Fig. 6), most likely, as mentioned, can be ascribed to the influence of vasodilator metabolites accumulating in the tissue as a result of the resistance increase and hence, reduced blood flow to the muscle.

Beta-adrenergic effects on segmental vascular resistances

It may be concluded from the results presented above that, in the adrenergic control of the vascular bed of skeletal muscle, the α -adrenergic component overrides the β -adrenergic one in the resistance vessels, leading to a net increase in total regional vascular resistance above the control level. On the other hand, the vessels which control the size of the functional capillary surface area, i.e. precapillary 'sphincters' or terminal arterioles, seem to be dominated by the β -adrenergic control mechanism. This suggests that the β -adrenergic control is mainly confined to the microvessels. The problem was analysed by a different approach, viz. by observing segmental resistance responses to adrenergic stimulation (IV). This technique (see Methods) permitted separation of resistance responses occurring in 'proximal arterial vessels' (larger than about 30 μ m, i.d.), in the 'microvessels' (smaller than about 30 μ m), and in the 'large bore venous vessels'.

Such experiments, performed in the absence and presence of regional β -adrenoceptor blockade, gave the following main results. In the situation of unmasked α -adrenergic influence (i.e. after β -blockade), vasoconstrictor fibre stimulation and adrenaline and noradrenaline infusions caused pronounced constrictions in all three segments of the vascular bed (IV). With intact β -adrenoceptors, however, a clearcut β -adrenergic dilator component was revealed in the microvessels in terms of an attenuation of the α -adrenergic resistance response, most pronounced for adrenaline which virtually cancelled the α -adrenergic constriction (Fig. 3, paper IV). A similar, but smaller, β -adrenergic influence was noticed in the venous resistance section, but was not discernible in the proximal arterial vessels (cf. Table I, IV). Yet, β -adrenoceptors might be present also in the proximal arterial vessels, as revealed by dilator effects of the agonist isoprenaline, but the microvessels showed much greater sensitivity to this agent (Fig. 5, paper IV). These data seem to corroborate the opinion of a preferential β -adrenergic dilator control of the microvessels in muscle.

Beta-adrenergic control of capillary hydrostatic pressure

The fact that hemorrhage leads to net transcapillary absorption of extravascular fluid from skeletal muscle into the blood stream implies that a transcapillary driving force for fluid absorption is established. It should be recalled that lymphatic drainage from the muscle was prevented in these

experiments and that, therefore, the described fluid transfer is a true transcapillary event. The average effective transcapillary driving force for the hemorrhagic fluid absorption was calculated in approximate terms by dividing the observed rate of net fluid absorption by the concomitantly determined CFC value.

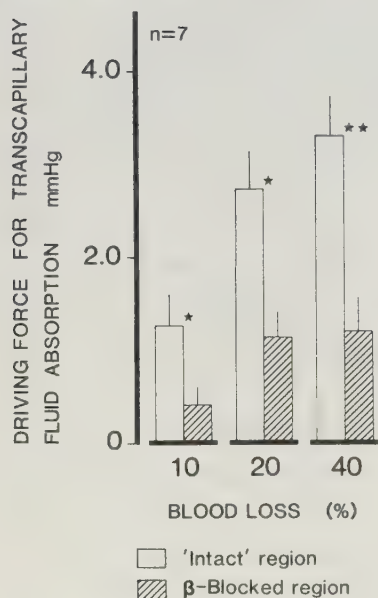


Fig. 7. Calculated data on the driving force for hemorrhagic fluid absorption in the muscle preparation with intact (white columns) and blocked (hatched columns) β -adrenoceptors. The data refer to the steady state 8-10 min after acute withdrawal of 10, 20, and 40% of the total blood volume.

Such data are shown in Fig. 7 for the 'intact' and β -blocked region after 10, 20, and 40% acute reductions of the animal's total blood volume. The data refer to observations obtained about 10 min after the acute blood loss. It can be seen that the calculated driving force for fluid absorption was significantly greater in the intact than in the β -blocked region at all levels of bleeding. This indicates that the β -adrenergic mechanism, in addition to its enlarging effect on the functional capillary surface area, contributes to the establishment of the driving force for fluid absorption in hemorrhage. The contribution to this driving force by the glucose-osmotic mechanism in hemorrhage was calculated to be similar before and after regional β -adrenoceptor blockade and was estimated not to exceed 1 mmHg in any of the experiments presented in Fig. 7. Since the colloid-osmotic driving force for fluid absorption can be considered to be largely maintained, at least not increased, during short term bleeding, the fluid absorption most likely is the result mainly of a fall of capillary hydrostatic pressure, P_c , causing a disturbance of the normal Starling fluid equilibrium. Since, as described in section 1, P_c was found to be well autoregulated in response to the

hemorrhagic fall of arterial pressure per se and since venous outflow pressure was kept constant, the decrease in P_c in hemorrhage can be ascribed to an active resetting of the pre- to post-capillary resistance ratio, R_a/R_v .

From these considerations, viewed in the light of the data in Fig. 7, the conclusion can be drawn that β -adrenergic mechanisms contribute significantly to the establishment of the increased R_a/R_v during bleeding. The data in Table 1 provide some information about the extent to which this occurs. This table shows calculated data for R_a , R_v and R_a/R_v derived from the hemorrhage experiments before and after β -adrenoceptor blockade illustrated in Fig. 1, assuming in the calculation an average mean P_c value of 15 mmHg in the isovolumic control period prior to bleeding (for ref. see Järhult & Mellander 1974). The estimated value for the glucose-osmotic driving force for fluid absorption was taken into account in the calculation of the P_c change during bleeding.

Table 1. Changes of precapillary (R_a) and postcapillary (R_v) vascular resistances, and of R_a/R_v evoked by acute hemorrhage (Fig. 1) in skeletal muscle with intact and blocked β -adrenoceptors.

		R_a	R_v	R_a/R_v
'Intact' preparation	Control	9.9	0.93	10.6
	Hemorrhage	13.4 (+35%)	0.99 (+6%)	13.5 (+27%)
β -Blocked preparation	Control	10.9	0.98	11.1
	Hemorrhage	16.6 (+52%)	1.44 (+47%)	11.5 (+4%)

It can be seen that, on the β -blocked preparation with unmasked α -adrenergic influence, hemorrhage led to increases in both R_a and R_v , but roughly to the same relative extent in both sections, implying only a moderate increase in R_a/R_v (from 11.1 to 11.5). With intact β -adrenoceptors, the hemorrhage evoked increases in R_a and R_v were less pronounced due to β -adrenergic inhibitory interaction with the α -adrenergic constriction. This β -adrenergic dilator influence, however, was more effective, in relative terms, on R_v than R_a , resulting in a reinforcement of the R_a/R_v increase (from 10.6 to 13.5). This seems to be the main explanation of the greater fall in P_c on the intact than the β -blocked preparation, in turn contributing to the faster absorption rate in the former than the latter situation (Fig. 1).

It could be argued that the described difference in postcapillary resistance in part might be a passive effect of a rheological change of venous blood viscosity or of collapse of the venous vessels by lowered transmural pressure in bleeding. At the very low flow rates encountered during hemorrhage, especially on the venous sides, the shear rate might reach values which cause significant increase in venous blood viscosity and hence venous resistance (cf. Järhult & Mellander 1974, Öberg, Little, Rippe & Folkow 1975, Schmid-Schönbein 1981). Such an effect would be expected to be more pronounced on the β -blocked region where the unmasked α -constrictor influence leads to a lower blood flow than on the 'intact' region. In an attempt to separate such active and passive effects on postcapillary resistance, the muscle region was perfused at constant blood flow to maintain the flow velocity approximately constant. Under the conditions of constant flow and constant venous outflow pressure, changes of arterial inflow pressure reflects the change of total vascular resistance, and the change of P_c reflects more or less selectively the change of R_v (cf. Friedman 1973), implying that the effects on R_a and R_v can be separated. The change of P_c , as described above, was estimated from the observed net transcapillary fluid movement, divided by CFC.

Table 2. Effects of β -blockade on precapillary (R_a) and postcapillary (R_v) responses evoked by adrenaline infusions (n=12) and by sympathetic stimulation (n=11) in constant flow perfused cat skeletal muscle.

	Adrenaline infusion 5-7 $\mu\text{g}\cdot\text{min}^{-1}\cdot\text{kg muscle}^{-1}$		Nerve stimulation 3-4 Hz	
	Intact region	β -Blocked region	Intact region	β -Blocked region
Increase in R_a	11.9 $\pm$ 1.3	16.1 $\pm$ 1.5 *	13.7 $\pm$ 1.7	14.5 $\pm$ 1.5
Increase in R_v	0.3 $\pm$ 0.08	1.1 $\pm$ 0.10 ***	0.3 $\pm$ 0.05	0.5 $\pm$ 0.08 *

Such tests were performed on the muscle preparation during adrenaline infusions and sympathetic nerve stimulation (IV). The results are summarized in Table 2. In the β -blocked region, adrenaline caused pronounced α -adrenergic increases in both R_a and R_v . Both these responses were much attenuated on the 'intact' region due to an active β -adrenergic dilator interaction with the α -adrenergic constriction. It should be noticed that, in relative terms, the β -adrenergic dilator effect was more pronounced in the postcapillary than the precapillary resistance section, lowering R_v by 72% (from 1.1 to 0.3 resistance units) and R_a by 26% (from 16.1 to 11.9 resistance units). Similar

effects were found on the 'intact' and β -blocked region in response to sympathetic nerve stimulation, but the neural β -adrenergic dilator effects were smaller than those of adrenaline (Table 2). These experiments, in which passive rheological effects were largely excluded, thus strongly suggest the existence of an active β -adrenergic dilatation of the postcapillary resistance vessels in skeletal muscle.

Role of active tone in the postcapillary resistance vessels in the β -adrenergic control of transcapillary fluid absorption

It is obvious that no vasodilator action can be exerted unless the vessels exhibit an active tone upon which the dilator mechanism can act. Resting tone is normally much higher in precapillary than postcapillary resistance vessels in skeletal muscle, in the former due to the combined effects of myogenic and α -adrenergic mechanisms, whereas in the latter it might mainly be dependent upon the α -adrenergic influence, like in the capacitance vessels (e.g. Mellander & Johansson 1968). In the present experiments it was demonstrated that, in the resting sympathectomized muscle preparation, the basal tone in postcapillary resistance vessels was quite small, as evidenced by little, or no, decrease in R_v in response to a supramaximal dose of papaverine in constant flow experiments (cf. Diana 1970). Under these circumstances it was further shown that the administration of the β -adrenoceptor agonist, isoprenaline, caused no significant dilation of the postcapillary resistance vessels, unless their tone was first raised experimentally, for instance by the application of an α -adrenoceptor agonist, such as phenylephrine. This is in agreement with a report by Abboud, Eckstein & Zimmerman (1965). This finding helps to explain the fact that isoprenaline under free flow conditions caused a net transcapillary filtration of fluid into the sympathectomized muscle tissue, thus apparently due to a more or less selective active decrease in R_a , and hence decrease in R_a/R_v , with an increase in P_c as the result. This implies that the described β -adrenergic control of transcapillary fluid absorption in hemorrhage might be effective only if there is a simultaneous excitatory influence on the vasculature, especially in the postcapillary resistance vessels. Such excitation would seem to be assured by the concomitant α -adrenergic influence on the vessels in bleeding. The fact that adrenaline is especially effective in the control of the absorption process in bleeding might thus be related to its dual α - and β -adrenergic influence on the vasculature in skeletal muscle (see General Discussion).

COMMENTS

The present studies on adrenergic effects in the vascular bed of skeletal muscle permitted analysis of responses in the proximal arterial, the microvascular, and the postcapillary resistance vessels, as well as in the capacitance vessels and the precapillary 'sphincters'. From the observations of adrenergic activation in the absence and presence of β -adrenoceptor blockade it appears that the smooth muscle in all these sections of the vascular tree is supplied with both α - and β -adrenoceptors, although their relative abundance seems to be distinctly different. The functional analyses thus suggested a relative preponderance of β -adrenoceptors in the precapillary 'sphincters' or terminal arterioles which determine the size of the functional capillary surface area, and a relative preponderance of α -adrenoceptors in the other sections, especially in the proximal arterial vessels and the capacitance vessels. The reflex sympatho-adrenal control of the overall resistance function in the vascular bed is usually dominated by its excitatory α -adrenergic component although quite effectively attenuated by its inhibitory β -adrenergic component. The microcirculation with seemingly more abundant β -adrenoceptors appears, however, to be the main site of β -adrenergic control. This opinion was recently confirmed by Marshall (1982) using a vital microscopic approach.

The primary aim of this series of experiments was to elucidate the microcirculatory events underlying the β -adrenergic control of transcapillary fluid absorption in hemorrhage. The results indicated that this control is accomplished by two main effects. First, it contributes to the establishment of a decreased capillary hydrostatic pressure (P_c), implying that the roughly maintained colloid osmotic driving force for absorption leads to net transfer of fluid from the extravascular to the intravascular compartment. This fall of P_c can be ascribed to a resetting of the pre- to postcapillary resistance ratio, in turn caused by a β -adrenergic dilator action which, in relative terms, is more pronounced in the postcapillary than the precapillary section. Second, the β -adrenergic control causes relaxation of the precapillary 'sphincters' which results in a markedly increased functional capillary surface area available for fluid exchange, thus significantly promoting the fluid absorption process in bleeding instituted by the fall of P_c .

There are two main methods used for estimation of functional capillary surface area in whole organ studies, i.e. the CFC method and the PS (permeability

surface) method. The former method was employed here since it provides direct measurement of the capillary fluid filtration capacity. Further, this method is considered to provide the most reliable estimate of changes of precapillary 'sphincter' activity, because filtration in patent capillaries is largely blood flow independent (see Folkow & Mellander 1970). The PS-method provides a measure of the capillary diffusion capacity which is a problem beyond the scope of the present investigation. Mention should be made, however, that several researchers have reported a decrease in the PS-value in skeletal muscle in hemorrhage (e.g. Appellgren & Lewis 1972, Dahlberg 1979) indicating an impaired diffusion exchange despite the fact that the capillary surface area is enlarged, at least in short-term bleeding, as indicated by the present CFC data. These results may not be conflicting, but may merely reflect that the blood flow distribution within the capillary network can become inhomogeneous during bleeding (e.g. Eriksson & Lisander 1972, Amundson, Jennische & Haljamäe 1980), apparently due to preferential 'shunting' of blood through low-resistant capillary channels. This would result in impaired overall diffusion capacity (e.g. Renkin 1968), but not necessarily in impaired filtration capacity, since capillary diffusion exchange is much more blood flow dependent than capillary filtration exchange. Such a deleterious diffusion effect in bleeding might mainly be ascribed to strong α -adrenergic constriction, cell aggregation, or other rheological phenomena. It would appear that the β -adrenergic dilator effects in the microcirculation could be beneficial in this situation. This is suggested, indirectly, by the finding that the diffusion capacity in skeletal muscle, determined by the PS method, is reported to increase in response to adrenaline or isoprenaline infusions (Sheehan & Renkin 1965, Gosselin 1966).

4. CLASSIFICATION OF BETA-ADRENOCEPTORS IN THE MICROCIRCULATION OF SKELETAL MUSCLE

The main aim of the experiments described in the foregoing was to evaluate the principal role of β -adrenergic versus other regulatory mechanisms in the control of the fluid absorption process and associated vascular events in skeletal muscle during hemorrhage. Such a purpose justified the use of the non-selective β -adrenoceptor blocking agent, propranolol, in the analysis. It was applied locally to the studied muscle region in such a way that there were no systemic effects.

Several reports indicate that β -adrenoceptors in blood vessels can be either of the β_1 - or β_2 -subtype and, further, that both may co-exist in the same vessel (e.g. Furchgott 1976). There are some reports indicating that, in skeletal muscle, the β -adrenergic vascular control is mediated solely by β_2 -adrenoceptors (e.g. Cullum, Farmer, Jack & Levy 1969, Taira, Yabuuchi & Yamashita 1977). This opinion, however, was based only on studies of β -adrenergic control of total flow resistance in muscle, which poorly reflects the microcirculatory organization.

The aim of this investigation (V) was to determine whether the β -adrenergic control of the microcirculatory events responsible for transcapillary fluid absorption is mediated by β_1 - or β_2 -adrenoceptors. The experimental observations focused on β -adrenergic effects in the muscle preparation evoked by sympathetic stimulation and by catecholamine infusions. In this analysis, metoprolol ($500 \mu\text{g} \times \text{kg bwt}^{-1}$ i.v., see Åblad, Carlsson & Ek 1973) was used for 'selective' β_1 -adrenoceptor blockade. Compound H35/25 ($2 \text{ mg} \times \text{kg bwt}^{-1}$ i.v., see Levy & Wilkenfeld 1969) and compound IPS 339 ($300 \mu\text{g} \times \text{kg bwt}^{-1}$ i.v., see Imbs et al. 1977) were used for 'selective' β_2 -adrenoceptor blockade. Prenalterol (Carlsson et al. 1977) was administered close arterially in doses up to $50 \mu\text{g} \times \text{min}^{-1} \times \text{kg muscle tissue}^{-1}$ to accomplish 'selective' β_1 -adrenoceptor stimulation and salbutamol (Cullum et al. 1969) was administered close arterially in doses up to $20 \mu\text{g} \times \text{min}^{-1} \times \text{kg muscle tissue}^{-1}$ to accomplish 'selective' β_2 -adrenoceptor stimulation.

Table 3 summarizes the average increases in microvascular resistance (R_{micro}) and in CFC evoked in the steady state by regional sympathetic stimulation (5 Hz) and by regional infusion of noradrenaline ($1.5 \mu\text{g} \times \text{min}^{-1} \times \text{kg}^{-1}$) in the muscle preparation with intact and 'selectively' blocked β_1 - and

β_2 -adrenoceptors. Corresponding data on the adrenergically evoked net transcapillary fluid absorption, as determined after the full development of the initial capacitance response, are also shown. It can be seen that with intact β -adrenoceptors, sympathetic nerve activation raised R_{micro} by 7.6 resistance units, increased CFC by 58%, and caused net transcapillary fluid absorption at a rate of $0.085 \text{ ml} \times \text{min}^{-1} \times 100 \text{ g}^{-1}$. Blood-borne noradrenaline caused similar effects. These vascular responses were not significantly altered after blockade of the β_1 -adrenoceptors. Beta₂-blockade, on the other hand, caused a clear-cut augmentation of the neural and noradrenaline induced increases in R_{micro} and attenuated significantly the CFC response and the net transcapillary absorption of extravascular fluid.

Table 3. Effects of sympathetic stimulation and noradrenaline infusion on microvascular resistance, CFC, and rate of fluid absorption in skeletal muscle with intact and 'selectively' blocked β_1 -(metoprolol) and β_2 -(H35/25)-adrenoceptors.

	'Intact' region	β_1 -Blocked region	β_2 -Blocked region
NERVE ACTIVATION			
Increase in R_{micro} $\text{mmHg} \cdot \text{ml}^{-1} \cdot \text{min} \cdot 100\text{g}$	7.61 ± 1.19 (n=10)	7.20 ± 1.16 (n=10)	$10.99 \pm 1.07^*$ (n=8)
Increase in CFC, %	58 ± 9 (n=12)	54 ± 13 (n=12)	$16 \pm 9^{**}$ (n=12)
Fluid absorption $\text{ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$	0.085 ± 0.012 (n=12)	0.082 ± 0.012 (n=12)	$0.033 \pm 0.009^{**}$ (n=12)
NORADRENALINE			
Increase in R_{micro} $\text{mmHg} \cdot \text{ml}^{-1} \cdot \text{min} \cdot 100\text{g}$	5.70 ± 1.16 (n=9)	5.80 ± 1.20 (n=8)	$11.40 \pm 1.26^{**}$ (n=8)
Increase in CFC, %	89 ± 14 (n=11)	81 ± 17 (n=11)	$36 \pm 11^{**}$ (n=11)
Fluid absorption $\text{ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$	0.072 ± 0.010 (n=11)	0.081 ± 0.016 (n=11)	$0.040 \pm 0.007^*$ (n=11)

These findings, taken together, indicate that the β -adrenergic dilator responses in the microcirculation of skeletal muscle evoked by sympathetic nerve stimulation and blood-borne noradrenaline are mediated via activation of β_2 -adrenoceptors. Such receptors are also responsible for the microvascular dilator responses to adrenaline infusion, as evidenced in a few analogous experiments.

Further support for a β_2 -specificity of the microvascular β -adrenoceptors was obtained from experiments in which the 'selective' β_1 -adrenoceptor agonist, prenalterol, and the 'selective' β_2 -adrenoceptor agonist, salbutamol, were infused i.a. to the muscle preparation. Salbutamol evoked dose-dependent dilator responses in the microvascular resistance vessels and in the precapillary 'sphincters' on the preparation with intact β -adrenoceptors. These effects were abolished after β_2 -adrenoceptor blockade, but unaffected by β_1 -adrenoceptor blockade. In contrast, prenalterol caused no microvascular dilatation on the preparation with intact β -adrenoceptors.

COMMENTS

This investigation strongly indicates that the microvascular dilator responses in muscle to blood-borne and nerve released noradrenaline and to adrenaline are mediated by activation of β_2 -adrenoceptors. The blood-borne catecholamines are likely to activate 'humoral', non-synaptic receptors distributed in the media of the vessel wall. Also nerve released noradrenaline might activate such 'humoral' β -adrenoceptors after diffusion of the transmitter into the media (cf. de la Lande 1975, Bevan, Bevan & Duckles 1980). Activation of 'non-synaptic' β -adrenoceptors by nerve released noradrenaline was recently suggested to occur in adipose tissue (Belfrage, Fredholm & Rosell 1977). Preliminary experiments in our laboratory supported a similar mode of activation in the microcirculation of skeletal muscle as evidenced by analyses of the adrenergic neurogenic and humoral microvascular dilator responses, using cocaine as inhibitor of the neuronal re-uptake of noradrenaline and compound U-0521 as inhibitor of COMT. It cannot be excluded, however, that a specific postjunctional 'neurogenic' β -adrenoceptor located close to the varicosities may be present as well. However, such a 'neurogenic' noradrenaline receptor might a priori be expected to be a β_1 -receptor (cf. Lands et al. 1967), unless adrenaline acts as a co-transmitter as suggested, for instance by Rand, Majewski, McCulloch & Story (1978). Whatever the case, the present data indicate that the β -adrenergic dilator control of precapillary 'sphincters' and of the microvascular resistance vessels, which facilitates the hemorrhagic fluid absorption process in skeletal muscle, is mainly linked to the activation of β_2 -adrenoceptors.

The question may still be raised whether these β -adrenoceptors indeed are located specifically in the vascular smooth muscle, as assumed above, or if the dilatation possibly could be an indirect consequence of β -adrenergic

alterations of skeletal muscle metabolism (cf. Lundholm & Svedmyr 1965, Durán & Renkin 1976). Our results provide no answer to this since long debated problem, but current views derived from in vitro studies on isolated vascular smooth muscle seem to strongly favour the first-mentioned alternative.

Mention should finally be made that the presynaptic β -adrenergic facilitation of transmitter release from the adrenergic nerve endings (see section 1) most likely was interfered with by β -blockade in the present experiments. Yet, as mentioned, such a facilitating effect is considered to be relatively small and, after β -blockade, would have led to an attenuation of the observed α -adrenergic increase in microvascular tone due to decrease in the amount of released transmitter. The demonstrated postsynaptic β -adrenergic dilator influence thereby would have been underestimated, if anything.

5. β_2 -ADRENERGIC CONTROL OF COMPENSATORY PLASMA VOLUME RESTITUTION IN HEMORRHAGE

Our observations on the muscle preparation in the cat have demonstrated that hemorrhage is associated with considerable fluid absorption from the extravascular to the intravascular compartment, a process mediated to a major extent by β_2 -adrenergic effects in the muscle microcirculation. It was postulated by extrapolation (Results, section 1) that such β -adrenergic net fluid gain into the circulatory system, if occurring in the entire skeletal muscle mass, would contribute importantly to the compensatory plasma volume restoration in bleeding. The validity of such extrapolation was tested in this series of experiments.

The aim of this study was to evaluate the extent to which β_2 -adrenergic control mechanisms contribute to the overall plasma volume regulation in bleeding. This was done by direct determinations of plasma volume restitution after standardized graded hemorrhage on anesthetized cats in the absence and presence of systemic β_2 -adrenoceptor blockade in the circulatory system. The employment of the highly selective β_2 -adrenoceptor blocking agent, ICI 118,551 (Bilski et al. 1980) was essential for this analysis to avoid any interference with β_1 -adrenergic effects on cardiac performance.

Comparative observations were made on four groups of animals, viz. on cats with intact or blocked β_2 -adrenoceptors exposed to rapid withdrawal of 10 or 20 ml of blood per kg bwt, corresponding to about 20 and 40% reductions of total blood volume. Data on plasma volume, arterial hematocrit, and estimated blood volume were obtained in the control period prior to, and at 1 and 2 h after, bleeding. The prehemorrhagic control data did not differ significantly in the four groups (Table 1, VI), and were approximately as follows: Plasma volume $34 \text{ ml} \times \text{kg bwt}^{-1}$, blood volume $50 \text{ ml} \times \text{kg bwt}^{-1}$, and arterial hematocrit 34%, which is in agreement with previous reports (e.g. Groom, Rowlands & Thomas 1965).

Fig. 8 shows the extent of plasma volume restitution ($\text{ml} \times \text{kg bwt}^{-1}$) in cats with intact and blocked β_2 -adrenoceptors determined 1 and 2 h after the initial reduction of the blood volume by 10 and 20 $\text{ml} \times \text{kg bwt}^{-1}$, corresponding to an initial decrease in plasma volume by 6.6 and 13.2 $\text{ml} \times \text{kg bwt}^{-1}$ (hematocrit 34 %). It can be seen from panels A and B that plasma volume increased in both the intact and the β_2 -blocked cats after bleeding, but the

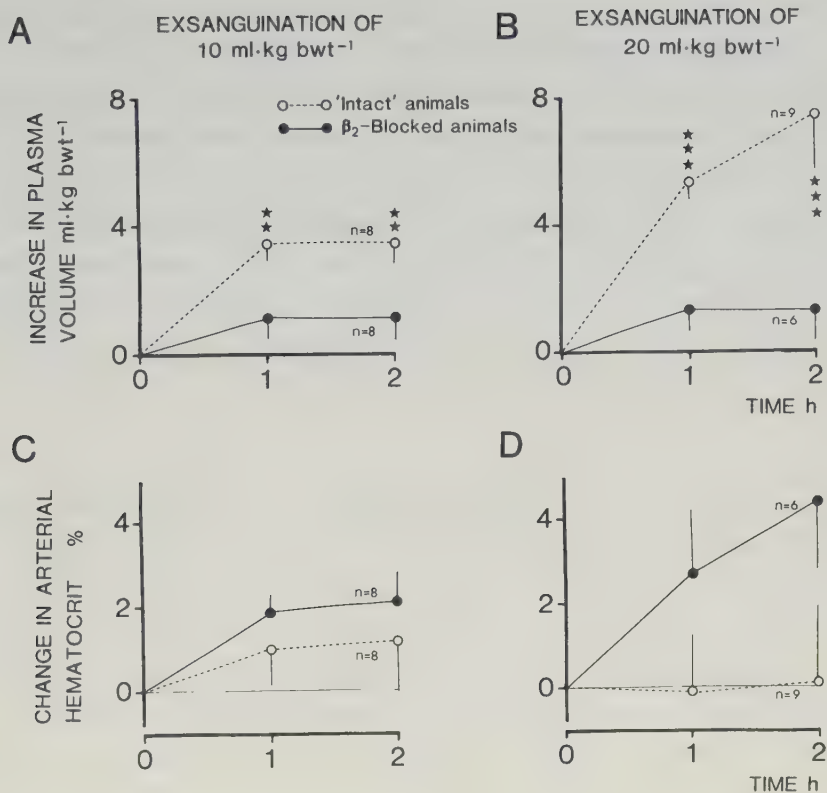


Fig. 8. Restitution of plasma volume (panels A and B) and changes of arterial hematocrit (panels C and D) after acute graded hemorrhage in animals with intact (open circles) and systemically blocked (closed circles) β_2 -adrenoceptors. The data indicate an important β_2 -adrenergic control of plasma volume restoration in bleeding.

increase was significantly greater in the former. With intact β_2 -adrenoceptors, the plasma volume restitution was 3.4 ml x kg bwt⁻¹ (52%) at both 1 and 2 h after the smaller blood loss (panel A) and 5.4 and 7.5 ml x kg bwt⁻¹ (41 and 57%) at 1 and 2 h after the more pronounced bleeding (panel B). In the β_2 -blocked animals, however, the plasma volume restitution was only 1.1 and 1.4 ml x kg bwt⁻¹ (17 and 11%) at these two degrees of bleeding. These data demonstrate that β_2 -adrenergic control mechanisms indeed are of great quantitative importance for the compensatory plasma volume restitution after blood loss.

The changes in arterial hematocrit observed in response to hemorrhage are shown in the lower panels of Fig. 8. In cats with intact β_2 -adrenoceptors, hematocrit tended to increase somewhat in response to the

smaller blood loss (panel C) but was unchanged after the more pronounced one (panel D). In the β_2 -blocked animals, there was a significant gradual increase in hematocrit at both levels of hemorrhage corresponding to 2.0 and 4.4%, respectively, 2 h after exsanguination. The fact that hematocrit remained unchanged or even increased in the face of plasma volume restitution indicates a concomitant increase in circulating red cell volume. This effect was mainly caused by recruitment of red cells from the spleen contracting in response to hemorrhage, as evidenced by the findings that, after acute splenectomy, bleeding ($10 \text{ ml} \times \text{kg}^{-1}$) caused a 7% decrease in hematocrit in cats with intact β_2 -adrenoceptors and that it was no longer increased after β_2 -blockade.

COMMENTS

The data presented in this section demonstrate an efficient compensatory control of plasma volume after acute hemorrhage, which within 1-2 h corresponds to a restitution of some 50-60% of the initially shed plasma volume, of which the major part (some 75%) can be ascribed to β_2 -adrenergic control mechanisms. This figure for plasma volume replacement is of similar order of magnitude to that arrived at by extrapolating our data on fluid absorption in the small muscle preparation to the whole muscle mass of the cat (section 1). The extrapolated figure, in fact, predicted a somewhat greater plasma volume increase than that actually found by direct determination. This suggests that the fluid absorption rate may not be identical in all skeletal muscles, or that some of the absorbed fluid is lost by extravasation into other tissues with time (see General Discussion). The post-hemorrhagic plasma volume replacement remaining after β_2 -adrenoceptor blockade (Fig. 8) most likely is explained by fluid absorption from muscle caused by the α -adrenergic and glucose-osmotic mechanisms. The data, taken together, are compatible with the view that the main mobile fluid reservoir for plasma volume replenishment in bleeding is that present in the extravascular space of skeletal muscle, in agreement with other reports (e.g. Öberg 1964, Eliassen et al. 1974, Järhult 1975, Reed & Wiig 1981).

Support for the opinion that the described β_2 -adrenergic plasma volume control in bleeding was caused by activation of postsynaptic β -adrenoceptors in the vascular bed of skeletal muscle and not by other β_2 -adrenergic effects (e.g. in the central nervous system) was presented in papers V and VI. The existence of a β_2 -adrenergic chronotropic control of the heart has been demonstrated by Carlsson, Ablad, Brändström & Carlsson (1972). Such an effect would have been

eliminated in the present experiments after β_2 -adrenoceptor blockade. Yet, the tachycardia response to standardized hemorrhage in vivo was found not to be smaller in the presence than in the absence of β_2 -blockade (Gustafsson, Hillman & Lundvall 1982) which might be ascribed to stronger sympatho-adrenal activation in the β_2 -blocked animals with impaired plasma volume restitution. Such a difference would have led to an underestimation, if anything, of the peripheral β_2 -adrenergic contribution to the described plasma volume restitution in hemorrhage.

GENERAL DISCUSSION

Acute hemorrhagic hypovolemia is met by a number of physiological defense mechanisms for survival of the organism which, within limits, are capable of restoring cardiovascular homeostasis. If these limits are surpassed, which certainly may be the case if the acute blood loss exceeds some 50% of the total blood volume (e.g. Rawson, Chien, Peng & Dellenback 1959), or if secondary circulatory derangements or other complications occur, these compensatory mechanisms alone are insufficient, and irreversible shock may ensue. Rapid compensation is of vital importance to avoid circulatory insufficiency.

A central role in the acute defense is played by the sympatho-adrenal system activated via cardiovascular mechanoreceptors, arterial chemoreceptors etc. Via its prompt control of cardiac performance, of the vascular resistance and capacitance functions, and of transcapillary fluid mobilization from the extravascular to the intravascular space, this system is instrumental for survival in the early stages of bleeding. Of these adrenergic adjustments, the compensatory plasma volume expansion caused by fluid absorption is the effect which most directly contributes to the early reestablishment of a temporary circulatory steady state until complete restitution is accomplished by the more gradual recovery of the entire body fluid balance and of the red cell volume.

Skeletal muscle is known to be the main target for the adrenergic control of transcapillary fluid absorption (for ref. see Mellander & Johansson, 1968). The present study demonstrates that this absorption process in bleeding, in addition to its previously established α -adrenergic and glucose-osmotic regulatory mechanisms, is most importantly governed by a β -adrenoceptor control of the muscle microcirculation. This β -adrenergic regulation causes an increased pre- to postcapillary resistance ratio lowering the capillary hydrostatic pressure, and a relaxation of the precapillary 'sphincters' increasing the size of the functional capillary surface area available to fluid absorption. This control, shown to be mediated via β_2 -adrenoceptors, seems to be responsible for a major part of the early hemorrhagic plasma volume restitution which, within 2 h, in total comprised 50-60% of the initially shed plasma volume. Both the neural and hormonal links of the sympatho-adrenal system contribute to the regulation of the fluid absorption process and both exert their actions, to a significant extent, via β -

adrenoceptors. The sympathetic neural control of the process is, however, transient in nature, whereas the hormonal control was shown to be maintained over extended periods of time, implying that the humoral catecholamines are of special importance for the prolonged cumulative fluid gain into the circulatory system in bleeding.

Discussion relevant to the interpretation of the present results was presented in the Comments sections in the foregoing. A few aspects relating more to the overall regulation of the cardiovascular system deserve some further attention.

It appears that the β -adrenergic, α -adrenergic, and glucose-osmotic regulatory mechanisms operate in concert to promote transcapillary fluid absorption from skeletal muscle in hemorrhage. It might a priori seem surprising that the direct neurogenic control of the fluid absorption process, although highly efficient per se, is such a relative transient phenomenon, being subjected, within few minutes, to an 'autoregulatory escape from vasomotor fibre influence' despite maintained constrictions in the resistance and the capacitance vessels (cf. Mellander 1960, Lundvall & Järhult 1976). A reason for this organization might be that the neurally induced fluid gain is a component participating also in more generalized cardiovascular response patterns, for instance in the everyday blood pressure control or in similar adjustments to circulatory stress other than hypovolemia. Such rapid neurogenic transcapillary fluid replenishment would then tend to reinforce the effect of the concomitant capacitance vessel constriction on venous return and cardiac filling. If, however, the neurogenic fluid absorption in such situations were long-lasting, untoward hypervolemia would result. In primary hypovolemia, on the other hand, when effective replacement of a true volume deficit is critical for survival, the control of the fluid absorption process in muscle must be maintained for considerable length of time. This requires additional regulatory mechanisms for fluid absorption, effected by the adreno-medullary control system and the glucose-osmotic absorption mechanism. The β -adrenergic dilator actions on precapillary sphincters and postcapillary resistance vessels exerted by the humoral catecholamines, especially by adrenaline, seem to be essential for the prolongation and facilitation of the absorption process in bleeding.

Another seemingly puzzling feature is that the vascular bed is exposed concomitantly to the opposing effects of α -adrenergic constriction and β -

adrenergic dilatation. One clue to the understanding of this dual control in hemorrhage might be that neither selective α -adrenoceptor, nor selective β -adrenoceptor, control would seem to guarantee a maintained regulation of the fluid absorption process, which apparently requires their mutual cooperation. This is related to the suggested uneven distribution of the α - and β -adrenoceptors in the vascular bed and to the complexities inherent in the control of the capillary hydrostatic pressure fall in bleeding. The latter is accomplished by an active increase in the pre- to postcapillary resistance ratio. The α -adrenergic mechanism no doubt causes an increase in the ratio, but its effect on fluid absorption appears to be transient. The β -adrenergic mechanism with its preferential dilator effect on the postcapillary resistance vessels requires, to cause an increase in the resistance ratio, the existence of a finite tone in these vessels upon which the dilator mechanism can act. Such tone was found to be small in the absence of extrinsic excitatory stimuli and therefore appears to be significantly related to an α -adrenergic constrictor influence in vivo, like the tone of the venous capacitance vessels. It should be recalled in this context that selective β -adrenoceptor stimulation by isoprenaline usually caused net fluid filtration into the sympathectomized muscle tissue, and not absorption, apparently due to the low basal tone in the postcapillary vessels in the control state after sympathectomy. The same filtration effect was previously reported for adrenaline when applied in such low doses that a net β -adrenergic dilatation in the overall resistance vessels was the result (Mellander 1960). It thus appears that, in vivo, the α - and β -adrenergic interaction on the pre- and postcapillary resistances is such that the beneficial fluid absorption is maintained in bleeding. In hemorrhage the α -adrenergic mechanism increases both resistances, and the β -adrenergic mechanism decreases both but, in relative terms, the former is more effective in the precapillary and the latter more effective in the postcapillary section, resulting in an increased resistance ratio and a consequent decrease in capillary hydrostatic pressure. It is noteworthy that a single control factor, like adrenaline, is designed to exert both these actions. Such fine a control of the resistance ratio can, however, be disturbed in states of seriously impaired tissue nutrition by the action of accumulating vasodilator metabolites which, via preferential inhibition of tone in the precapillary resistance vessels, can lead to detrimental plasma fluid loss into the tissues (Mellander & Lewis 1963, Lundgren, Lundwall & Mellander 1964).

Since the β -adrenergic control of capillary hydrostatic pressure and fluid

absorption seems to be effectively exerted only in the presence of some α -adrenergic influence on the vascular bed its importance would have been difficult, or impossible, to reveal by utilizing α -adrenoceptor blockade as a pharmacological tool in the present experimental approach. This justifies the use of β -adrenoceptor blockade, instead, to evaluate the β -adrenergic contribution to the process.

Mention may be made of a recent report by Marshall (1982) in which she claimed from vital microscopic estimations of diameter changes in selected venules of the rat spinotrapezius muscle, combined with a few histochemical observations, that venous smooth muscle entirely lacks sympathetic innervation. From such findings on this specialized flat skeletal muscle in the rat, with its rather uncommon vascular arrangement, the author drew the apparently far-reaching conclusion that venous smooth muscle in general, including that of the capacitance vessels, has no active neural adrenergic vasomotor control whatsoever in any skeletal muscle of other species, like the cat and the dog. Marshall's opinion is interesting, but obviously in direct conflict with established facts presented from numerous laboratories. It appears from her discussion that this deduction rested on several misinterpretations of the data of other researchers. It seems clear from the present results that the vasoconstrictor nerves do control both the postcapillary resistance vessels and the venous capacitance vessels of limb skeletal muscle in the cat. The α -adrenergic veno-constrictions are to a significant extent actively induced, though in many cases, like in the present bleeding experiments, reinforced by passive elastic recoil of the venous vessels due to precapillary constriction, as clarified by Mellander (1960) and subsequently by several others (for ref. see e.g. Chien 1967, Mellander & Johansson 1968). However, the postcapillary constrictions, as mentioned in the foregoing, are much smaller in absolute terms than the precapillary ones and apparently are difficult to reveal on a single venule observed under the microscope. This can be exemplified by the fact, that Marshall was unable to detect the luminal reduction caused by passive elastic recoil in the venules that must have occurred, according to herself, during the reported strong arteriolar constrictions in response to paravascular nerve fibre stimulation. The paravascular nerves stimulated in that study may further not contain all adrenergic fibres distributing to the various sections of the vascular bed of the spinotrapezius muscle.

The fluid absorbed from skeletal muscle in hemorrhage most likely contains little or no plasma proteins and therefore would lead to lowered plasma

colloid osmotic pressure with time, as also shown by direct measurements (e.g. Skillman, Awward & Moore 1967). Recovery of plasma protein concentration from hepatic or extravascular pools apparently is a slow process. It might be questioned, then, why the absorbed fluid is retained within the circulatory system, as shown by the present observations of plasma volume restitution, and not lost by secondary ultrafiltration into the tissues. This might be explained, at least partly, by the action of the glucose-osmotic mechanism which, besides its effect on plasma volume restitution, seems to contribute to the overall regulation of extracellular fluid volume in hemorrhage. Thus, after transcapillary distribution into the interstitial space of all tissues, glucose creates an interstitial hypertonicity leading to osmotic fluid withdrawal from the intracellular compartment (Järhult 1975, Ware, Nordberg, Norman & Nylander 1982). Such intracellular protein-free fluid would tend to dilute the plasma proteins in the entire interstitial space, thereby, contributing to a maintenance of an effective colloid osmotic driving force for fluid absorption across the capillary membranes.

The ultimate aim of the sympatho-adrenal compensatory adjustments in hemorrhage is to restore nutritional blood flow to the tissues. The β -adrenergic control system seems to contribute to such an effect both via its dilator influence on the microcirculation and via its influence on central hemodynamics caused by plasma volume restitution. The latter problem was recently approached by studying directly the influences on central hemodynamics in hemorrhage exerted by β_2 -adrenergic peripheral vascular control mechanisms (Gustafsson, Hillman & Lundvall 1982). In this investigation, central hemodynamic responses evoked by acute withdrawal of 40% of the total blood volume were followed during a 2 h observation period in cats with intact and systemically blocked vascular β_2 -adrenoceptors, using the 'selective' β_2 -blocking agent, ICI 118,551. During the first 10 min after bleeding, blood pressure and cardiac output decreased and total peripheral resistance increased to roughly the same extents in both groups of animal. Later on, arterial pressure stabilized at the same hypotension level in both groups, but the other hemodynamic variables were distinctly different. In the animals with intact β_2 -adrenoceptors there was a gradual, partial recovery of stroke volume and cardiac output parallel to a return of total peripheral resistance to the prehemorrhagic control level. In the β_2 -blocked animals, however, total peripheral resistance continued to increase in the face of a maintained low cardiac output and declining stroke volume. The lower stroke volume in the latter group could be ascribed to the abolition of the β_2 -

adrenergic restitution of plasma volume. The gradual decline of total peripheral resistance in the 'intact' animals was to a significant extent due to the β_2 -adrenergic dilator interaction with constrictor influences on the resistance vessels, and partly an effect of intravascular refill. The β -adrenergic vascular control mechanisms thus help to improve nutritional tissue blood flow during hemorrhage by increasing plasma volume and, hence, venous return and cardiac output, and by decreasing peripheral resistance, the latter effect also contributing to decreased after-load on the heart. In addition, they induce dilatation in the microcirculation. These physiological reflex β_2 -adrenergic circulatory events are similar to those aimed at in shock therapy by transfusion and vasodilator treatment.

If the results presented here can be shown to apply to man, they might have important clinical implications, for instance in view of the common use of β -blockers in the treatment of cardiovascular disease. Studies aimed at disclosing a possible β -adrenergic control of the muscle microcirculation in humans are in progress.

SUMMARY

The present investigation deals with the transcapillary fluid absorption process in skeletal muscle during hemorrhage, its underlying control mechanisms, and its functional significance for posthemorrhagic compensatory plasma volume restoration. The study, performed on anesthetized cats, provides evidence for the opinion that β -adrenergic vascular control mechanisms contribute importantly to the fluid absorption from muscle in response to standardized rapid blood loss. This was revealed by comparison of the rates of fluid absorption, determined by continuous measurements of change of tissue volume in the lower leg muscle preparation, in the absence and presence of regional β -adrenoceptor blockade (propranolol).

The absorption rate to a standardized blood loss was thus about three times greater on preparations with 'intact' than with blocked β -adrenoceptors indicating that the process was governed, to a major extent, by β -adrenergic mechanisms, reinforcing the effect of the previously established α -adrenergic and glucose osmotic regulation of posthemorrhagic fluid absorption. The β -adrenergic control of the fluid absorption was strictly graded in relation to the extent of bleeding, tested for 10, 20, and 40% reductions of the animal's total blood volume.

Both the neural and the hormonal links of the sympatho adrenal system contribute to the control of the fluid absorption process in bleeding and both exert their effect, to a significant extent, via a β -adrenergic mechanism. The neural control is rapid in onset but seems to be relatively transient in nature. The hormonal control by blood borne catecholamines is more gradual but well maintained over an extended period of time implying that it is of special importance for the long-term fluid gain into the circulatory system in bleeding.

The mechanisms underlying the β -adrenergic control of the fluid absorption process were analysed by detailed studies of the reactions in consecutive vascular sections in the muscle preparation with intact and blocked β -adrenoceptors in response to bleeding and other types of adrenergic activation. These analyses referred to observations of resistance in the whole vascular bed, in its precapillary and postcapillary sections, and in its proximal arterial, microvascular, and large bore venous segments. Observations of the capillary filtration coefficient permitted estimation of changes in the

functional capillary surface area. Capacitance responses were recorded volumetrically.

Such observations led to the conclusion that the β -adrenergic control in skeletal muscle is mainly confined to the microcirculation and that it governs the fluid absorption process in hemorrhage by evoking a decrease in capillary hydrostatic pressure and a simultaneous increase in functional capillary surface area available for fluid exchange. The capillary pressure fall is accomplished by a resetting of the pre- to postcapillary resistance ratio, in turn caused by a β -adrenergic dilator interaction with concomitant α -adrenergic constrictor influences on the resistance vessels, and this dilator effect, in relative terms, is more pronounced in the postcapillary than the precapillary resistance section. The increase in functional capillary surface area can be ascribed to a β -adrenergic relaxation of the precapillary 'sphincters', which significantly promotes the fluid absorption process in hemorrhage.

The β -adrenergic postjunctional microvascular dilator responses were analysed with regard to β_1 - or β_2 -adrenoceptor specificity with the aid of 'selective' receptor agonists and antagonists. The conclusion was reached that the neural as well as the humoral β -adrenergic control of the microcirculation in muscle is exerted via activation of β_2 -adrenoceptors.

Direct determination of the plasma volume restitution after acute blood loss showed that 50-60% of the initially shed plasma volume was replaced within 2 h in cats with intact β -adrenoceptors. After systemic adrenoceptor blockade with a highly 'selective' β_2 -antagonist (ICI 118,551), the plasma volume restitution was reduced to some 25% of that observed with intact β_2 -adrenoceptors showing that the plasma volume compensation, to a major extent, is mediated via β_2 -adrenoceptors. These data for plasma volume replenishment were of the same order of magnitude as arrived at by extrapolating the data for fluid absorption in the muscle preparation to the whole skeletal muscle mass in the cat. This indicates that the fluid absorption from muscle is mainly responsible for the overall compensatory plasma volume restitution occurring within the first few hours after acute blood loss. It is concluded that the described β_2 -adrenergic control of transcapillary fluid absorption and plasma volume is of great hemodynamic importance for the maintenance of cardiovascular homeostasis in early stages of bleeding.

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